CIBA FOUNDATION COLLOQUIA
ON AGEING

Vol. I. General Aspects

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# CIBA FOUNDATION COLLOQUIA ON AGEING

## VOLUME I

## General Aspects


*Editors for the Ciba Foundation*

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*Assisted by*

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*With 38 Illustrations*





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THE CIBA FOUNDATION

*for the Promotion of International Co operation in Medical and Chemical Research*

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# PREFACE

THE Ciba Foundation's international conferences are arranged as often as possible at times when the members can on the same journey also participate in other appropriate conferences in Western Europe. As early as 1952 Professor R. E. Tunbridge asked whether the Ciba Foundation would be willing to hold a suitable conference near the time of the third Congress of the International Association of Gerontology, to be held in London in July 1954, and to this the Director of the Foundation readily agreed.

Early in 1954 the Trustees of the Ciba Foundation decided to embark on special measures in support, internationally, of basic research relevant to the problems of ageing, so that the conference already arranged became the first in what it is hoped will be a series of conferences on subjects in this field. In conformity with the Foundation's earlier series of "Colloquia on Endocrinology", the new series is being called "Colloquia on Ageing", whilst the conferences on isolated subjects will continue to be described as 'Symposia'.

In the arrangements for this colloquium on the General Aspects of Ageing the Director was greatly helped by the advice he received from Professor Tunbridge, who also kindly undertook the duties of Chairman.

The colloquium was intended to be a general exploration and appreciation of the present position in regard to opinion and experiment on the processes associated with or directly involved in the changes occurring in tissues with age, at whatever period of life of the organism from conception to death.

To those to whom this book serves as an introduction to the activities of the Ciba Foundation it should be explained that it is an [illegible]

It owes its inception and support to its founder, CIBA Ltd of Switzerland, but is administered independently and exclusively by its distinguished British Trustees

The Foundation provides accommodation for scientific workers who visit London from abroad organizes and holds international conferences, conducts (in conjunction with the Institut National d'Hygiene) a postgraduate medical exchange scheme between England and France, arranges informal meetings for discussions awards an annual lectureship has initiated a scheme to encourage basic research relevant to the problems of ageing (as mentioned above) assists international congresses and scientific societies, is building up a library service in special fields and generally endeavours to give aid in all matters that may promote international co operation in scientific research

Leading research workers from different countries and in different disciplines are invited to attend the symposia or colloquia The size of the group is however, very strictly limited in order to obtain a free conversational manner of discussion—although the basic timetable of the programme is strictly observed The smallness of the groups means the exclusion of many workers active and interested in the subjects discussed and therefore the proceedings of these conferences are published and made available throughout the world

It is hoped that the papers and discussions in this book will prove not only informative and stimulating but will also give to readers a sense of participation in an informal and friendly occasion

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List of those participating in or attending the Colloquium on Ageing: "General Aspects," 13th–15th July, 1954.

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| A. VON ALBERTINI | Histopathological Inst., University of Zürich |
| J. C. AUB | Massachusetts General Hospital, Boston. |
| SIR F. C. BARTLETT | Applied Psychol. Research Unit, Cambridge. |
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# CHAIRMAN'S OPENING REMARKS

R. E. TUNBRIDGE, O.B.E., M.D., F.R.C.P.

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FIRST of all I know that it is your wish that I should thank the Ciba Foundation, on your behalf, for giving us this opportunity of meeting together to discuss the problem of ageing. The Director wisely chose as the title of the Symposium "Ageing—General Aspects". We have thus been spared any etymological discussion on what is the *meaning of* gerontology. During the course of our discussions we shall wander from the most general aspects of the problem to the particular, but I trust that even when we are discussing minutiæ we shall keep in mind the general problem of ageing. Perhaps it is too much to hope that at the end of this Conference we shall be able to define ageing precisely and clearly—but possibly many of us would feel that a lot of fun had gone out of life if the riddle were to be solved so easily.

Is ageing a chronological term merely reflecting the passage of years, and if so what years, or are the public right in assuming, as they generally do, that ageing is synonymous with senescence and/or decay? The concept of the elixir of life, like the philosophers' stone and the alchemists' dream of the transmutation of metals, has long served as a tremendous stimulus to mankind, to higher flights of imagination or sometimes to derision. We shall not dwell upon these fantasies, nor shall we deal with that other very important aspect of the problem, what one might call the political, economic and social aspects of ageing, of which we as citizens cannot be unaware. We shall try, I hope, to confine ourselves to the more biological aspects in an attempt to define, if it is possible, the process of ageing.

Is there an allotted span of life? Is there species specificity with regard to the life span? Is the length of life determined

by the structure of the chromosome? We can breed animals with longevity as a dominant, and it is well known that there are human families in which longevity is a striking characteristic This is a fascinating aspect of the problem, Professor Medawar has very strong views on this approach to ageing, and we look forward to hearing his lucid exposition

Then again, is senescence itself a primary biological function? Is it inevitable or is it merely the predominance of catabolism over anabolism? Is it a feature of cellular life, of the life of an organ or of an organism? Alexis Carrol in his experimental work suggested that environmental factors were highly important, if not the most important factor in senescence or the changes which we associate with senescence Then we have the problem of the cyclic changes that occur in many organs, for example the thyroid or the ovary Is the hypertrophy and involution a fundamental biological process, and is senescence allied to it, an exaggeration of it, or a similar process in another phase? I understand that the senescent or atrophied ovary of an adult animal can be transplanted into a young ovariectomized animal of the same species, and that it then proceeds to take on cyclic function again Therefore, is it merely the milieu in which the organ lives, the environmental factors, which determine age changes, or are they separate and distinct processes? We have with us experts like Dr Betty Rubin Dr Parkes, Professor Krohn who may not deal with this particular problem, but who as a result of their pre eminence in the field of endocrinology are bound to touch on similar topics

Then, of course, there is the extraordinary power of organs or tissues to regenerate We are familiar with the great powers of regeneration of, say, the liver, and not merely the growth but the readiness with which new cells take on apparently normal function, whereas in other tissues, like the nervous system, once the nerve cell is seriously damaged it is no longer capable of repair We have with us many experts on the changes in tissues with ageing Are the changes in the skin the arteries, the bone, the lungs, just senescence? Are they

specific? Is senile osteoporosis, so common in elderly women, merely a manifestation of osteomalacia in a particular age group or is it a specific degenerative change? It is interesting that elastic tissue so abundantly laid down in youth seems to cease to be laid down, or only sparingly so, in old age, its place being taken by collagen. We have to decide on the relationship of these problems to the whole problem of ageing: the riddle is interminable, and possibly our own approach is coloured. I hope in our discussions we shall keep this in mind and that we shall leave this colloquium invigorated and refreshed.

The programme also refers to aspects of nutrition, and you will notice that because of the question of bias we have not neglected to include the psychiatric and psychological aspects of the subject. Here the field is less well defined, and Professor Aubrey Lewis and Professor Sir Frederic Bartlett might say we are all highly biased and therefore truth is very difficult to come by.

# THE DEFINITION AND MEASUREMENT OF SENESCENCE

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## Introduction

Nothing is clearer evidence of the immaturity of gerontological science than the tentative and probationary character of its system of definitions and measurements. 'What is ageing?' is a question to which there is no agreed answer. It is therefore very natural that the first contribution to this colloquium should be an attempt to suggest the ways by which an answer might be arrived at.

'Ageing', in the literal sense of merely growing older, might well be a beneficial process, i.e. there are certain reasons why an animal's expectation of further life should increase instead of becoming shorter with advancing years. Animals grow wiser as they grow older, so far as memory can influence their actions: the survivors of earlier hazards are less likely to be their victims when exposed to them for a second time. For example, Lack (1951) has shown that beyond the first few months of life the mortality of wild birds is independent of their age; but game birds (whose census is partly carried out by gunfire) may suffer a disproportionately high mortality during the first two years of life, after which the survivors presumably know better. Then again, animals become wiser in an immunological sense, for antibody formation is a process with a long memory, and a second response to an antigenic stimulus is much more effective than the first. (Immunologists were speaking of the anamnestic response—a not forgetting of past behaviour—long before biologists came to regard the storage of information as a conceptual novelty.)

Yet ageing in man is accompanied by that general deterioration which the word itself colloquially implies, and the same

is true of all mammals and birds which have been artificially kept alive long enough to suffer the inroads of senile decay The deterioration that goes with ageing could be described as a decline of 'vitality' or a lowering of biological efficiency—of an organism's power to maintain itself as a going concern but this is more of an innuendo than a formal definition and the question remains how is senescence to be measured and defined with acceptable precision? (It will be convenient to use the seventeenth century word *senescence* to stand for the deterioration that accompanies 'ageing' and to leave 'ageing' itself to stand for merely growing old )

The process of senescence and the state of senile deterioration can be measured or assessed in two entirely different ways (Medawar 1952) First there is the kind of measure that may be called *personal* because it purports to measure a process that takes place in the life history of an individual animal second there is a statistical or actuarial measure which is founded upon the mortality of a population of individuals and which bears only indirectly upon the changes that occur within the lifetime of anyone The former is used by the pathologist physiologist and (by an extension of his terms of reference) the embryologist The latter is used by biologists generally and in particular by ecologists and by students of the genetical theory of evolution

## The Personal Measure of Senescence

If we knew of some master factor or prime mover in the process of senescence—if for example every ingredient of decay was causally dependent upon changes occurring in the pineal gland—then a personal measure of senescence would be in principle entirely adequate The one master process could be measured and the rest should follow But we know of no such master factor the processes of senescence are in gear but we do not know which is the driving wheel All that can be done therefore is to record separately the several manifestations of decay—the wrinkling of the skin and greying of hair the anatomical involution of the organs the

growing insufficiency of endocrine output, the loss of acuity of the senses, the weakening of muscular power and coordination, the decline of the specific growth rate and rate of cellular turnover, and so on. All these can be described and measured in fact or in principle, but as mere description cannot allot causal priorities among their competing claims, no one of them can be held to be *the* measure of senile decay.

Considered one by one, each of these measurements gives different estimates of the time of onset and rate of progress of senescence. Take, for example, the specific growth rate of the body, i.e. its rate of growth considered as a system increasing by compound interest, in which that which is formed by growth is itself capable of growing. Many years ago Charles Minot pointed out that the specific or percentage rate of growth in man fell from birth onwards, rapidly at first, and then progressively more slowly. Man grows like money invested at a rate of compound interest which falls progressively at a rate which itself progressively falls. Minot argued that the specific growth rate was a particularly searching and intimate measure of vitality; it follows that senescence begins at birth and goes on faster in children than in their elders. Estimates of the rate of healing of wounds have been held to reveal a similar trend, but some of the data will not stand up to ill-disposed criticism.

Measurements by other standards give different answers. Acuity of hearing is said to be sharpest at about the age of ten and resistance to infectious disease to be at its maximum at about fifteen. Muscular power and coordination are presumably at their peak at about age twenty-five. If therefore we think of the body as a multiplicative growth system, there is a sense in which senescence could be said to begin at birth, as an antibody-forming system, at fifteen and as a muscular machine at twenty-five. These several measures of vitality or senescence are therefore incongruent, and it is an important empirical fact that they are so; but for the time being we must concede that no one measure of senescence based upon the properties of individual organisms

can yet claim causal precedence over any other, and we must therefore try our luck elsewhere

## The Actuarial Measurement of Senescence

The actuarial measurement of senescence is based upon the following presupposition that although senescence is indeed a diverse and complex process, with *manifestations* in all the structures and activities of the body, its net total

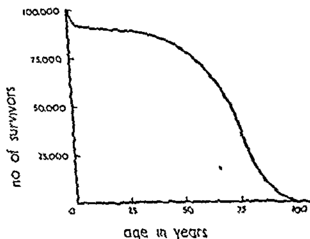



FIG 1

*effect* is of one measurable kind—to increase vulnerability, the *likelihood of dying* as life goes on

A measurement of vulnerability *is implicit* within the information set out in an actuary s *life table* A life table may be thought of as a series of *figures* representing the surviving residue *year by year* of a *population* marked at birth and followed through *life until all* have died The table therefore begins with some *arbitrary but* large number of individuals ($l_0$, say 100 000) *and* ends at zero when none survive In the form of a graph a *life* table for human beings has the shape illustrated by Fig 1

The curve in Fig 1 represents the falling away of the number of survivors ($l_x$) in each year $x$ of age Its slope is the death rate, i e *the rate of decline of the number of survivors* where it is steep the death rate is high were it horizontal the death rate would be zero The slope is downward and therefore has a negative sign, to make the death rate positive, it may be written

$$\text{death rate} = -\frac{dl_x}{dx}$$

The death rate itself is almost useless as a measure of the likelihood of dying for its numerical value clearly depends upon the number of individuals still left to die (*The death rate at 100 is lower than at any previous age because so few* are left in the running ) What is required is the age specific death rate or force of mortality the death rate at any age divided by the number still exposed to hazard, i e

$$\text{force of mortality} = -\frac{1}{l_x}\frac{dl_x}{dx},$$

the negative sign being used as before, to give the force of mortality a positive value In a population of which the members do not deteriorate with increasing age—e g a population of plates or test tubes—the likelihood of being broken is no greater at any one age than at any other at the end of each age interval the number of survivors falls to a constant fraction of its value at the beginning of the interval the force of mortality is therefore constant The curve of the force of mortality in human beings has the shape illustrated by Fig 2 *It is high at birth falls steeply to a very low value between* the ages of eight and fourteen and then rises without singularity or inflexion for the remainder of life

The question now is can the force of mortality be used as a measure of the degree of senescence over that period of life in which the force of mortality is increasing? (There is no question of its being used for such a purpose during that earlier epoch of life in which its value is falling )

The difficulties and covert implications of such a scheme of measurement may now be commented upon one by one

(1) In the first place senescence is a process At their face value the life table and its derivatives record not a sequence of events which takes place in the lifetime of a single individual but the age frequency distribution in the population as a whole of a single event in life viz death its end

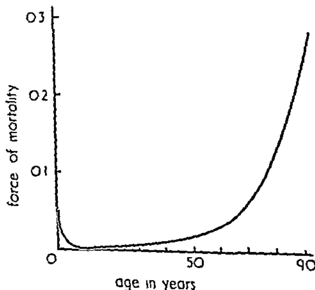



FIG 2

This is not a weighty criticism It is a well understood convention that with certain safeguards the frequency of the occurrence of an event in a population can be taken to represent the probability of its happening to an individual If these safeguards are observed (not all can be see below) we can legitimately claim to be measuring that change in an individual's lifetime which makes him progressively more vulnerable to mortal hazards

(2) The use of the force of mortality assumes that all death is accidental in origin (using the word accidental in its

widest sense) and that senescence is the process of becoming ever more accident prone In other words everyone is assumed to die *of* something, no one to die what is most inaptly called a 'natural death Most pathologists believe this to be true (Aschoff among them) and all are obliged to do so by the laws of certification The approach to natural death is therefore asymptotic The lethal threshold falls until in theory, the most trivial accident imaginable is a cause of death

(3) The measurement is corrupted by the existence of causes of death which are not random in their incidence, i e to which the entire population is not equally at risk The barnacles of Hatton s (1938) life table* may well be equally exposed to hazard but human beings are obviously not special ages and occupations have special risks Evidently the measurement must be corrected for causes of death having any kind of specific frequency of incidence

(4) A life table set up by tracing the life histories of a cohort of newborns from birth to death will certainly be corrupted by *secular* changes in the causes of mortality Men aged fifty four today were born in 1900 when the nature of the causes of death and their pattern of incidence were very different from what they are today Animals reared in laboratories may not be so affected nor need it be assumed that secular errors of this kind affect wild populations of animals with comparatively short lives

(5) If the force of mortality is to be a reasonably efficient measurement of the degree of senescence there should be no gross inherited differences of susceptibility between members of the population at risk For let it be supposed that the population with which the life table begins is genetically subdivided into stalwarts and weaklings Selection will then occur in the course of life and the population which the later years of the life table deals with will be a quite unrepresentative sample of that with which it began In the laboratory

*For the literature of life tables for wild animals see Deevey (1947) Allee Emerson Park Park and Schmidt (1949) and Haldane (1953)

the use of inbred strains or their $F_1$ hybrids can eliminate this source of error

None of the five objections so far raised challenges the *principle* of using vulnerability as a measure of senescence, though they make it very evident that grave difficulties stand in the way of its practical use They are difficulties which apply with particular force to human beings—in this respect, as in so many others, a tiresome and anomalous animal, rightly excluded from the syllabus of studies in most departments of zoology We may now consider two genuine shortcomings of principle, one of which is reparable, the other not

The first is that senescence may reveal itself by a true and radical deterioration that is not accompanied by any increase in the likelihood of dying viz a deterioration of reproductive power Should not any measurement of senescence take reproductive capacity into account?

The question is pertinent, because in human females reproduction comes to an end over an epoch of life that is not distinguished by any dependent change in the likelihood of dying (On the contrary, one cause of death, in childbirth has been outlived ) It may have less bearing on other animals, in which reproduction, instead of stopping rather suddenly, merely slows down in step with other senescent changes A more f[illegible]

[illegible] p[illegible] its kind—should have regard to both mortality and fertility Several such measures suggest themselves of which the most useful might be the total future expectation of offspring per head *from* a chosen age $x$ onwards viz the reproductive value

$$\frac{1}{p_x}\int_x^\infty p_x b_x \, dx,$$

where $b_x$ is the age specific birth rate, and $p_x$ $(=l_x/l_0)$ represents the probability of living to age $x$ (or, in effect, the value of $l_x$ when $l_0$ is taken to be unity)

There is therefore no difficulty in coming to terms with this first objection of principle, if need be The second is of an entirely different kind

By the word 'senescence' we clearly wish to describe a change which is of innate origin which is built into the developmental structure of an animal and which could take place even under the most favourable' conditions it is *possible to envisage* The force of mortality does indeed measure this inborn decline, but the difficulty is that it must also measure another sort of decline of purely contingent origin as well

It cannot be strictly true to say, as I said earlier, that plates and test tubes are subject to a constant force of mortality, even when given equality of use and of exposure to the risk of being broken They suffer indeed no innate decline (if we disregard such refinements as the slow crystallization of glass) but they may deteriorate in a different way Plates and test tubes get cracked and chipped but may yet be usable, at least in the more frugal homes and laboratories A cracked test tube or plate is more vulnerable than one which is still intact, it is more likely to get broken in the course of ordinary use There is therefore, or may be an increase of vulnerability which has nothing at all to do with innate decline but which is due to the persistent, therefore cumulative, effect of recurrent injury or stress

There are innumerable examples in living organisms of an increase of vulnerability caused by the persistence and with time the summation of the effects of recurrent damage stress and biological error (cf Medawar 1952) Any injury which leaves any physical trace is an actuarial debt and will add to the likelihood of dying A single example will illustrate the distinction which may be drawn between deterioration of innate and of merely contingent origin the formation of ectopic flexure lines or creases in the skin, which (if the efforts made to conceal them are anything to go by) are among the most characteristic and reliable signs of the deterioration associated with increasing age

Ectopic flexure lines form on those parts of the body where the skin is habitually creased or folded particularly on the face A single creasing of the skin is insufficient to form an overt flexure line but presumably even one creasing leaves some trace in the dermal fibrous skeleton because if the skin is creased sufficiently often a flexure line will eventually appear Old people have acquired lined faces for two clearly distinguishable reasons (a) because an innate deterioration of their skin has made it more susceptible to the engravery of habitual usage and (b) because they have smiled or frowned or raised their eyebrows more often than their juniors There can be little doubt that the first factor the true innate ' deterioration of the skin by far outweighs the latter, and that it represents the process which most people understand by senile change but both agencies are at work the persistent and cumulative effects of recurrent stress and the progressive decline of power of the skin to resist its action

This is a trivial example but it would be idle to deny that purely contingent factors influence the vulnerability of animals particularly when the organ or part of the body that is affected is constitutionally incapable of repair For a higher animal to lose a limb may not in itself be fatal but if it does so it will certainly be more vulnerable to the hazards which limbs are normally used to escape from It may be that the actual life span of wild carnivores is as closely bounded by the life of their teeth as by any other single factor Teeth may become more fragile with increasing age but whether they do or not it is quite certain that one good reason why an old animal should have fewer sound teeth than a young one is because time alone unconnected with senile decay has given the older animal more opportunity to break them In short risks are not all of an all or nothing character there gra l ent of or

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are not in themselves mortal may build up a debt which is due to age, because it depends upon the passage of time, but which is *not* due, in the colloquial sense, to "ageing".

The two classes of agencies which contribute to increase of vulnerability need not be difficult to distinguish by experimental means, but in the actual records of mortality the two are inextricably combined If we wish to use an actuarial measurement of senescence, this is a real shortcoming of principle Yet this confounding of two causes of increase of vulnerability does not in any way detract from the *biological* value of the use of the force of mortality as a measure of senescence, for both are real agencies that govern the population dynamics of real communities of animals It is up to the physiologist and pathologist to distinguish and keep apart the contribution to vulnerability that is made by a true innate decline

In summary, the actuarial definition of senescence amounts simply to this: that senescence is a change which accompanies ageing and which makes an individual progressively more liable to die (Such a definition may be challenged on many grounds, but its simplicity should not be one of them ) Senescence may be measured by the force of mortality estimated from the age frequency distribution of the incidence of death, but the measurement is valid only of a genetically uniform population subjected to a constant pattern of hazards impingeing randomly upon it, and is imperfect in proportion as these conditions fall short of being fulfilled Increase of vulnerability is caused partly by an innate deterioration of the body and partly by the accumulation of an actuarial debt as a result of recurrent exposure to injuries that leave a persistent mark. The separation of the latter contribution from the former should depend upon the context in which the phenomenon of senescence is being studied.

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# SOME REMARKS ON THE PATHOLOGICAL BASIS OF AGEING

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*Department of Morbid Anatomy, University College Hospital Medical School, London*

Two years ago, two Swiss doctors, A. L. Vischer and F. C. Roulet (1952) reported the death of two of their countrymen at or near the age of one-hundred-and-two. Both of these old people had led healthy active lives, escaping serious illness and remaining free from any disability up to the end. Death came to the man as the result of gangrene of a limb after influenza, the woman faded out after the death of a daughter, bronchopneumonia being the immediate cause of death. Careful investigation of bodily functions in both towards the end of their lives failed to disclose any serious disturbance. Basal metabolism, recovery from fractures, plasma proteins, hæmoglobin index, blood cell counts, blood pressure and pulse were within normal limits.

Autopsy on the one hundred and two-year-old male disclosed generalized arteriosclerosis, especially severe in the abdominal aorta and lower extremities, with thrombosis of both femoral, popliteal and tibial arteries and of the accompanying veins, gangrene of the left foot, terminal bronchopneumonia and chronic pulmonary emphysema. Other findings included a carcinoid tumour in the lower ileum, regeneration nodules in the thyroid, and glandular and muscular increase in the prostate.

The old lady, almost one-hundred-and two years of age when she died, showed at post mortem examination cancer of the breast associated with cystic mastitis but no metastases, severe arteriosclerosis of the aorta, coronary disease with obstruction, calcification of the annulus fibrosis of the heart

and brown atrophy of the heart and liver cholelithiasis and a nodular goitre

To all intents and purposes therefore these two old people were healthy right up to the terminal illness yet their bodies were a collection of morbid processes any one of which might have flared up at any time during the last thirty years and ended life

No doubt an extended search of the pathological literature would bring to light further reports on extreme old age but I am not aware of such information so I set to work to collect data from the autopsy records of University College Hospital which bear on the topic of ageing and these I now present in the hope that they will interest you

Our records over the years 1904–1953 *comprise* 13 630 autopsies among which are 1 872 on old people Examinations were conducted by five pathologists who were, on occasions assisted by juniors however it is safe to claim that autopsy technique has altered very little during the fifty years for each of us with one exception has been trained by his predecessor In many instances microscopy on the most important organs was performed and such reports have been available for study I am greatly indebted to two of my research colleagues N Udall and K C Basu Mallik, who sorted out the cases for me

Reports on males from sixty five years onwards and females from seventy years onwards have been scrutinized Since there were very many more males than females (1 328 against 544) most of my remarks will be confined to the former

Let me say at once that all I have endeavoured to do is to find out what was the pathological condition responsible for the decline and death of the individual In many instances, of course the immediate cause of death was some terminal accessory event such as an acute infection a severe hæmorrhage septicæmia or uræmia It is therefore more correct to speak of morbidity rather than mortality I must first of all explain that our hospital is a teaching institution and I gather from conversations with our senior clinicians that the

policy for many years has been to admit as varied a type of patient as is possible from the population that closely surrounds us. Of course there will have been some selection and no one would dare claim that our material represents a random sample of the London or English population. We have had surgeons, for instance, who for a time were especially interested in some kinds of cancer for which they were engaged in perfecting techniques for treatment. Likewise we have attracted at one time or another, heart cases or patients referred to a lung or liver specialist. Caution must also be maintained in drawing conclusions from our ageing cases for they have been admitted to our hospital because they were suffering from some fairly obvious complaint. Against this can be placed figures such as those given by Drs Trevor Howell and A P Piggot of St John's Hospital, Battersea, whose material was still less likely to be selected than ours. These workers also give information collected by Dr G Stewart Smith in various municipal hospitals. Suffice it to say that there is reasonable agreement in general between the results obtained from the three independent series, although the classification and emphasis often differ.

In Tables I and II, I summarize the principal morbid conditions encountered during five-yearly intervals. In every instance I have carefully scrutinized the autopsy records and endeavoured to decide what condition dominated the complex pathological pattern at death. In practically every case I have agreed with the considered opinion of the pathologist responsible for autopsy. You will see from the tables that 70–80 per cent of deaths are included under the diagnosis of cancer, arteriosclerosis, urinary disease (especially senile enlargement of the prostate and its complications), primary lobar or bronchopneumonia and chronic peptic ulceration of the stomach or duodenum. In both sexes cancer and arteriosclerosis make up the majority of morbid conditions. Table III groups together the dominant conditions in the total groups. It turns out from these latter figures that 40 3 per cent of males die from cancer and 20 8 per cent die from

arteriosclerosis 31 per cent of females die from cancer and 27 4 per cent from arteriosclerosis Let me repeat once again that these pathological states in my opinion were the cause

Table I

THE COMMONER MORBID CONDITIONS FOUND AT AUTOPSY IN 1,329 MALES FROM SIXTY FIVE YEARS ONWARDS

Percentage and total cases

| Morbid Condition | Age Groups | | | | |
|---|---|---|---|---|---|
| | 65–70 | 71 75 | [illegible]6 80 | 81–85 | 80 |
| Cancer | 43 8% | 41 0% | 36 6% | 31 6% | [illegible]6 8% |
| Arteriosclerosis | 17 9 | 19 6 | 26 4 | 27 6 | 48 3 |
| Urinary disease | 6 7 | 9 0 | 8 4 | [illegible] 6 | 6 8 |
| Gastric and duodenal ulceration | 4 7 | 4 1 | 3 0 | 1 3 | 3 4 |
| Pneumonia | 3 7 | 3 3 | 7 4 | 15 8 | 13 6 |
| Total cases in groups | 635 | 306 | [illegible] | 76 | 29 |

Table II

THE COMMONER MORBID CONDITIONS FOUND AT AUTOPSY IN 544 FEMALES FROM SEVENTY YEARS ONWARDS

Percentages in total cases

| Morbid Condition | Age Groups | | | |
|---|---|---|---|---|
| | 70–75 | 76–80 | 81–85 | 86 |
| Cancer | 39% | 2[illegible]% | 18% | 21% |
| Arteriosclerosis | 19 | 31 | 41 | 47 |
| Pneumonia | 5 | 0 | 15 | 12 |
| Total cases in groups | 275 | 153 | 73 | 43 |

of decline and often of death of the subjects but that frequently enough an acute complication was the immediate cause of death

But some of these figures are misleading for all who have examined the organs of aged persons must have been struck

by the frequent association of a number of morbid conditions. In an attempt to assess this association I have re-examined with care, reports on 100 ageing male subjects from sixty-five years upwards suffering from cancer and compared them with autopsy reports on 100 subjects dying from causes other than cancer. All of these autopsies were performed by myself at University College Hospital during the years 1935-39 and

Table III

THE COMMONER MORBID CONDITIONS FOUND IN AGEING MALES (SIXTY-FIVE YEARS ONWARDS) AND FEMALES (SEVENTY YEARS ONWARDS)

Total numbers

| Morbid Condition | Males | Females | Total |
|---|---|---|---|
| Tumours mostly cancers | 555 | 178 | 733 |
| Arteriosclerosis | 277 | 149 | 426 |
| Digestive disease | 162 | 74 | 236 |
| Respiratory | 116 | 62 | 178 |
| Urinary | 108 | 14 | 122 |
| Nervous | 13 | 4 | 17 |
| Total cases | 1231 | 481 | 1712 |

Total number of subjects investigated 1872

1946 and 1947. Table IV gives the results. Without attempting to draw any quantitative comparisons I feel sure you will agree that the cancer cases show a very high incidence of coronary, aortic and renal arteriosclerosis, in other words of generalized arterial disease. Similarly the non-cancerous ageing subjects are very frequently affected with generalized arteriosclerosis. I believe that it is safe to conclude that cancer and arteriosclerosis are common morbid conditions in ageing persons and that they very frequently go together.

I have likewise looked into cases of chronic urinary disease, peptic ulceration and bronchopneumonia and I have found with these, too, that severe arteriosclerosis is very common. Since it is *much more difficult to collect adequate numbers* for analysis than with cancer, I have not attempted to present

figures, but I can assure you that my conclusion seems definite enough

Finally, I give you in Tables V and VI some details about the common types of cancer on the one hand and the distribution of arteriosclerosis on the other You will notice how certain sites are often picked out by cancer, especially stomach œsophagus, rectum and lung I might say that the list of possible sites is a long one, and the rarer examples are

**Table IV**

Arteriosclerosis in Cancer and Non Cancer Male Cases

| | 100 Cancer Cases | 100 Cases free from Cancer |
|---|---|---|
| Age range | 65–84 | 65–83 |
| Heart weight (g) range | 210–570 | 210–935 |
| Coronary disease | | |
| Normal | 8 | 3 |
| Slight | 42 | 32 |
| Moderate | [illegible] | 27 |
| Severe | 25 | 39 |
| Complete obstruction | 17 | 26 |
| Aortic | | |
| Normal | 3 | 0 |
| Slight | 28 | 28 |
| Moderate | 33 | 38 |
| Severe | 34 | 34 |
| Renal | | |
| Normal | 24 | 11 |
| Slight | 32 | 45 |
| Moderate | 32 | 32 |
| Severe | 8 | 5 |
| Pyelonephritis | 4 | 7 |
| Cerebral | | |
| Normal | 33 | 33 |
| Slight | 10 | 14 |
| Moderate | 5 | 16 |
| Severe | 5 | 12 |
| Not examined | 45 | 25 |

not included. So, too, with arteriosclerosis there is a predominance of certain types, especially coronary and the generalized type with its associated congestive cardiac failure.

Table V

TYPES OF CANCER AT DIFFERENT AGES IN MALES

| *Type of Cancer* | *Age Groups* | | | | |
|---|---|---|---|---|---|
| | 65–70 | 71–75 | 76–80 | 81–85 | 86– |
| Stomach | 49 | 24 | 15 | 3 | 1 |
| Lung | 38 | 27 | 4 | 4 | 0 |
| Rectum | 25 | 17 | 5 | 3 | 0 |
| Œsophagus | 35 | 17 | 7 | 2 | 0 |
| Colon | 22 | 8 | 12 | 0 | 1 |
| Bladder | 14 | 4 | 3 | 2 | 0 |
| Pharynx | 13 | 3 | 0 | 1 | 0 |
| Larynx | 10 | 5 | 6 | 1 | 0 |
| Prostate | 10 | 11 | 6 | 2 | 0 |
| Total cases | 287 | 150 | 74 | 22 | 2 |

Table VI

TYPES OF ARTERIOSCLEROSIS AT DIFFERENT AGES IN MALES

Total numbers

| *Types* | 65–70 | 71–75 | 76–80 | 81–85 | 86 |
|---|---|---|---|---|---|
| Coronary disease | 60 | 27 | 22 | 7 | 4 |
| Generalized AS with CCF | 22 | 24 | 10 | 4 | 6 |
| Cerebral thrombosis | 9 | 8 | 11 | 6 | 0 |
| Cerebral hæmorrhage | 16 | 8 | 9 | 3 | 3 |
| Aneurysm including syphilis | 10 | 5 | 1 | 1 | 1 |
| Total | 117 | 72 | 53 | 21 | 14 |

From all such scrutinies—and I must emphasize that both naked-eye and microscopic examinations have been employed in the vast majority of cases—I have reached the following conclusion. In every instance of ageing one or more well-defined chronic morbid condition can be found at work

in a number of organs Experience has taught us that these are serious processes capable enough of bringing about the death of affected individuals at any age. In many cases, it is true, equally serious acute, killing diseases are also present but they are of short duration and are merely complications. I have yet to encounter an instance where a clear-cut pathological basis could not be found and, moreover, one which had obviously progressed and become more dangerous as age advanced I cannot exclude the possibility that cases may be met with in which morbidity is not very obvious, but I feel more and more certain with increasing experience that rigorous investigation invariably brings to light pathological equivalents for ageing (I doubt very much whether there are specific structural disturbances due to old age, and that alone.) I hold the view that ageing is merely the vector sum of a number of morbid processes most of which take a time to develop, and often a long time to reach a serious climax. Many of these commence when we are young, perhaps even when we are born, and insidiously develop throughout the greater part of our active lives The remedy for ageing must needs be sought for in youth Death, then, constitutes one means of stopping the progress of morbidity With Montaigne I believe that 'Death is but an end to dying".

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DISCUSSION

*Tunbridge* We have had a tremendous contrast in approach in the last two papers, and I am ... now to attack ... Medawar abo... that there are ... the structure

that this was a dangerous generalization Surely regular usage is one of the features of our physical training and attainment of efficiency?

individual belongs to the group which will die under the 50 per cent probability of twe

which survive to c

colony A measur

standing One way we tried was by studying the adaptation capacity of an individual to low atmospheric pressure It turned out that there are some rats which adapt very well, while others, even at the age of twelve or fourteen months do not It might be possible that with such an activity measurement we can differentiate between those individuals which are biologically old and others which are biologically still young I should like to ask whether Prof Medawar thinks that such a method would lead to a measurement of individual biological age?

so to adapt the animal to the low pressure It sounds a simple and neat method of measuring what is obviously a profoundly important biological function

*Cowdry* I am very much interested in both Prof Medawar s and Prof Cameron s presentations and I think they afford an admirable background for any discussion on just what ageing is It's my impression that we all agree that every tissue and almost every cell ages differently from the next one that we are really a medley of different replacements and different ages that there are many histological tests for ageing as well as functional ones that the test of performance is really as good as, or better than, the test of vulnerability I am impressed, of course with the statements of Prof Cameron about cancer and arteriosclerosis and the pattern that is presented in these numerous autopsies here in London What strikes me is the probability that the pattern would be altogether different in some other countries I know that the cancer pattern shows geographical differences for instance, intra oral cancers in Bombay are very numerous indeed, and in Japan gastric cancers exceed all the other death dealing cancers Arteriosclerosis is rather conspicuous in England and in the United States, and is very much less so in certain Oriental races, and these two major variables change the whole picture or spectrum of age changes, it seems to me When we were discussing the organization of our book on problems of ageing the first question that we were asked to come to some agreement about was what is to be regarded as normal and what as pathological? We couldn't

to use the

information

whether it

is of individuals or of cells. Now, as Prof Medawar has pointed out, you can't get a similar population of human beings because their individual heredity is all different and the races are different. We can standardize

me it settles down with dignity, but if you take a young hand and do it, it snaps back. There, ladies and gentlemen, is one of the best histo-

much troubled by the severe mental disease of her daughter, was

[illegible] psychiatrist a few years ago visited all 12 centenarians who in that year

*Lansing* I was very much impressed with Prof Cameron's data, particularly in that they seemed to [illegible]

out earlier [illegible]

steadil

windin

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incide

wonder whether the arteriosclerosis case refers to the eighty five-year old individual, the rather rare cancer case at that age, or whether that refers to the individual of sixty five who is one of the 43 per cent who had cancer There may be a shift in your population But still further, [illegible]

slight or at least moderate arteriosclerosis There are no tumours, no gross lung changes, no gross liver changes, the prostates are normal, and so on My question is—does that rare individual die of what I think of as ageing? Does he exist at all in England?

*C*[illegible] cancer takes so many years to develop—you reach an age when all the cancer has developed which is likely to develop But your explanation may be better than mine

*Lansing* I had no explanation, I just raised the question But wouldn't the same then hold for arteriosclerosis, which has a heavy toll in midlife?

*Cameron* I don t feel happy about arteriosclerosis, about how long it takes to produce it But cancer, I feel, from the experimental work and so on is a thing where there is a time limit

*Lansing* Do you ever find that rare individual the 1 in 100 without pathological findings?

*Cameron* I d say that in 1 in 1000 one may fail to find them

*Lansing* I believe Dr Cowdry, you had a case some years ago?

*Cowdry* I didn t have the case, but there was a lady who died in Barnes Hospital aged one hundred and thirteen or thereabouts who was very agile and apparently in good health until just before she died There were certain police records she was arrested for practising medicine without a licence in Kansas City aged ninety five The charges however, were not preferred and she was let off She was thrown from a horse and kicked in the mouth when she was ninety something There are a great many details We have numerous cases of this remarkable phenomenon—some few people growing to extreme old age very gracefully and almost immune to the ravages of time Of [illegible] of the prematurely aged person and [illegible] factor There is also a [illegible] tissue fluids as well as [illegible] cells The question of [illegible] see if studies could now be made using radioactive isotopes, to discover the rate of turnover of these substances with age. It should be possible to secure quantitative data on the ageing of a considerable number of tissues without too

great difficulty Then we d have to resort to statistical analysis taking say 1 000 people of different ages, levelling it off by eliminating individual variations There might not be as great individual variations as we think in this turnover

*Brull* The main outlooks which have been pronounced here this afternoon about ageing are (1) compensation and (2) adaptability We know that we can lose a great deal of our main tissues and still compensate with what is left of them We stress too much the lesions we

*Cowdry* You could put it in a word couldn't you, by saying that it is the ability to maintain steady states in the tissue groups—homeostasis

*McCay* Not till they were
the epiphyses do
in the bones

retarded then the whole pathology seems to be pushed far out into another time area where it doesn't occur until the rat should theoretically be dead. And, of course, we need such tests as those Prof. Verzár has just described; it's extremely important that we have some means of [illegible]

*Schulze*: I would like to stress that it is most valuable to pay attention to the connection between premorbid functional conditions in the arteries and the development of morphological changes in arteriosclerosis of man, and to trace out this connection. About thirty years ago Burger proved that in the progress of ageing a steadily increasing deposition of organic and inorganic catabolites takes place in the so-called 'bradytrophic tissues', which have no or only a slight capillary supply and are nourished predominantly by diffusion. This was also [illegible]

degree of arteriosclerotic changes in human vessels is not identical on both sides of the body. There is some difference to be found between the right and the left side an[illegible]
a reasonably higher cont[illegible]
their vessels on the right [illegible]
there are marked and apparently systematic differences in the biochemical composition of arteriosclerotic vessels between the arms and the legs. In my opinion these facts are likely to demonstrate a close relationship between functional wasting processes and the incidence of arteriosclerotic changes. This idea should be borne in mind in further research work on the pathogenesis of human arteriosclerosis.

*Comfort*: I want to raise the question whether senescence of the endogenous type occurs in all vertebrates. It's a problem which the literature doesn't se[illegible]
and I'd welcome any [illegible]
remember that some [illegible]
*suggested that fish, which never cease to grow throughout life,* don't exhibit, or wouldn't exhibit if it were possible to construct their life table, the same sort of increase in the force of mortality with increasing age which you find in mammals. There are two distinct problems here. There is the group of senescent effects due wholly to accumulation of *injuries which Prof. Medawar mentioned, and there is the fact that it is* virtually impossible to devise direct experiments by which the force of mortality in these long-lived cold-blooded animals can be studied. [illegible]

ases to

great one ... that the work of Dr McCay is

... open our

...ntial

the

...rat

McCay I can't answer the basic problem at all though we have

*Moon H D Simpson M E, Li C H and Evans H M 1952 *Cancer Res* 12 448

retarded then the whole pathology seems to be pushed far out into another time area where it doesn't occur until the rat should theoretically be dead. And, of course, we need such tests as those Prof. Verzár has just described, it's extremely important that we have some means of measuring this potential towards long life and resistance to pathological changes so that we can go back into the genetics. We need criteria for animals that are going to live for very long periods.

*Schulze:* I would like to stress that it is most valuable to pay attention to the connection between premorbid functional conditions in the arteries and the development of morphological changes in arteriosclerosis of man, and to trace out this connection. About thirty years ago Bürger proved that in the progress of ageing a steadily increasing deposition of organic and inorganic catabolites takes place in the so-called "bradytrophic tissues" which have no or only a slight capillary supply and are nourished predominantly by diffusion. This was also proved for the inner layers of the aortic wall. Combining these careful analyses of the biochemical structure of the arterial walls Dr. Hevelke, a *colleague of mine at Prof. Bürger's clinic, recently pointed out that the* degree of arteriosclerotic changes in human vessels is not identical on both sides of the body. There is some difference to be found between the [illegible] ...ded men have [illegible] ...n the walls of [illegible] Additionally there are marked and apparently systematic differences in the biochemical composition of arteriosclerotic vessels between the arms and the legs. In my opinion these facts are likely to demonstrate a close relationship between functional wasting processes and the incidence of arteriosclerotic changes. This idea should be borne in mind in further research work on the pathogenesis of human arteriosclerosis.

*Comfort:* I want to raise the question whether senescence of the endogenous type occurs in all vertebrates. It's a problem which the literature doesn't seem very explicit about. I've been trying to find out and I'd welcome any suggestions that anyone here has to offer. You may remember that some years ago Bidder (*Brit. med. J.* 1932, 2, 583) suggested that fish, which never cease to grow throughout life, don't exhibit, or wouldn't exhibit if it were possible to construct their life [illegible]

[illegible]

tortoise of the Queen of Tonga, which is alleged to be very old and which [illegible]

*Franklin* I should like to suggest for consideration later that a definition of senescence is a decrease with time in the physiological reserves of tissue and in the physiological capacity to increase the number of cells

retarded again and kept in a state of suspended development, or was it all or none?

*McCay* I've never let them first become sexually mature and then stopped them You'd create another variable there, the variable of body-weight and adipose tissue We have taken rats half through life and then forced them to become very thin and again, "the thin rat goes to the funeral of the fat rat"

*Comfort* That was a rather separate effect, wasn't it?

*McCay* Yes, that's a separate effect that you run into as soon as you try that type of experiment beyond sexual maturity

*Comfort* I have a medical bias here because while it would be nice to keep patients in an arrested state as children as you kept your rats children, the real medical problem is to prolong the period of adult vigour And although, I suppose, restriction of diet is beneficial, you have no evidence so far that one can bring about the same slowing down of the record in adult rats that one can in young ones

*Olbrich*: Dr McCay, did you measure any type of function, say liver function, in your retarded rats for a prolonged period and correlate it with the time?

*McCay* No, I never did In our hands rats are difficult for functional measurements and we'd always hoped we could get dogs where we could make functional measurements

[illegible]

the rat, and we hope Prof Verzár's functional tests continue to develop and we'll apply them to the retarded rats

*Krohn* Have you any notion about what is happening that prevents actual maturation of the reproductive tract? Is there any particular reason why the gonadotrophins should fail to be produced in your own animals?

*McCay* No We've been trying to find out how late in life the rat can reproduce Asdell made initial studies on the œstrus cycle a long while ago, but now we are actually trying to breed them very late in life after they've long been retarded We are using those same rats for certain types of psychological testing at present We're also hoping to use parabiosis, and suture a retarded rat to a rat that's growing old

*Lansing* There have been a number of studies in lower organisms which show that retardation through dietary control is effective only prior to maturation during the period of growth in the *Cladocera* and

[illegible]

extension of the growth period will result in a 10 or 20 per cent increase in life-span

*Franklin* I should like to suggest for consideration later that a definition of senescence is a decrease with time in the physiological reserves of tissue and in the physiological capacity to increase the number of cells

# MENTAL ASPECTS OF AGEING

AUBREY LEWIS, M D, F R C P
*Institute of Psychiatry Maudsley Hospital London*

AGEING is a stage in the total process of growth and decay, *and it might be expected that its mental aspects like those of* childhood, would cover most of psychology, since age is a variable that can hardly ever be left out of account in any psychological inquiry But there is no such extensive literature about the latter end of life as there is about the earlier For this there are obvious sentimental, social and technical reasons In spite of the relatively meagre data it is not easy to survey the present stage of knowledge because it reflects changing theories and methods in regard to such fundamental matters as learning and personality

*Any general question as to the essential nature of ageing* does not admit of a psychological answer the question is a biological one and concerns the whole organism rather than only its psychological aspect I shall therefore limit myself to considering some questions about the manner in which we age mentally Chiefly these are Is there a differential rate and degree of decline in psychological abilities which may permit reorganisation and even gain just as it may entail disorganisation and decay? What are the characteristics which distinguish healthy ageing from pathological ageing? Are they differences only of degree? Are some mental changes of age compensatory and adaptive? What is the physical substrate of mental ageing? How far do social and cultural influences affect the ageing individual either by hastening and aggravating his handicaps or by allowing readjustments and opportunities that suit him?

Before seeing what can be said in reply to these questions I should like to look briefly at a concept which crops up almost as

often as adaptation or stress This is the concept of regression It would be more correct to say the concepts for behind this word there are evidently several meanings when it is applied to ageing The simplest is that originally given it by Ribot when he introduced the term to denote the retention in memory of the earliest perception and experiences whereas other memories are lost in the reverse order of their acquisition this is based on crude and on the whole erroneous observations but is at any rate a defined and appropriate use of the word Another commoner meaning refers to the way in which when a man is confronted with harsh experiences difficult or impossible to adjust to his personality r[illegible] or regresses to earlier levels of integration it has the

features of the personality nor how far back it [illegible] personality A third meaning of the term by no means open to these objections is perhaps open to others of a more [illegible] of the term

of why so many senescent people pay excessive attention to their bowels the more the genital zone retires into the background as a source of pleasure the more many individuals turn back to oral and anal erotism as in infancy Yet another meaning of regression is that which Kurt Lewin gave it he regarded various sorts of disorganisation and reduction of the field of activity as regressive, pointing out that the patterns of behaviour observable in senility or imprisonment strongly indicate that the different aspects of regression are to a certain degree independent of each other The term has yet other meanings in this context Spielmeyer used it for instance to describe the arterial changes in senile brains and of course in German psychiatry late middle life is called

[illegible]

But it may be said that beneath this variety there is one common notion, and an important one—that the ageing man reverts to the psychological ways of his earlier life. This is a very ancient and familiar idea: Aristophanes wrote that old men are boys again, and no doubt it goes much further back than that. But the "second childhood" view of old age derives too obviously from some superficial features of senile dementia. Old men, it is true, may become self-centred, greedy, boastful, prone to tantrums, sulky and in other ways "childish", as we say; but I see no more reason for equating this behaviour with the corresponding behaviour of a young child than for equating an old man's tottering gait with a youngster's toddle, or an old man's urinary incontinence with a baby's bedwetting. It is a purely descriptive similarity, whereas the concept of 'regression' implies essential identity. Unlike Hughlings Jackson's principle of dissolution of function, to which it has a deceptive affinity, it neither explains nor describes.

The psychoanalytical use of the term, however, cannot be criticised on these grounds. Granted the psychoanalytical theory, regression does in part describe the psychopathology of many of the emotional changes and morbid beliefs of the senescent or senile patient. It is almost entirely, however, a matter of inference and analogy: very few elderly people have had an actual psychoanalysis. And it fails us, as do so many concepts based on study of the abnormal, if we wish to account for the constructive readjustments which often appear in later life, in spite of reduced capacity in some respects.

The first general question, and the most difficult, is to distinguish the intrinsic or normal psychological accompaniments of chronological ageing from those that are morbid. The problems in this are not different from those we meet when we try to distinguish between physiological and pathological ageing, but I think they are incomparably more difficult to solve. Apart from the common trouble in defining any essential distinction between normal and morbid, we have very little ground for correlating mental with structural changes; we cannot isolate mental functions readily, and

many of the most important we cannot measure, study of animals takes us only a little way, and we cannot control some variables which are probably powerful influences upon the rate and signs of mental ageing in human beings Consequently we are deprived of many of the methods which are available for separating pathological from normal in the somatic features of old age

It might be said that these difficulties can evidently be overcome, since they have been overcome at the other end of life To some extent that is true We know a great deal about mental changes in children as they develop, but in them also the difficulty of telling what is pathological may arise in matters of emotion and behaviour, as the experience of every child guidance clinic attests In any case ageing is spread over a longer period than childhood is, its timing and form are probably less ordained in advance by heredity, and the strong environmental influences that may play upon it are harder to control for purposes of investigation Hence the greater obstacles in studying its normal character

If we could relate the normal mental characteristics of old age to structural changes in the ageing brain, we might be better off This we cannot do Such changes occur, of course, but have been very little studied in the normal The only thorough examination of ...
uncomplicated by deme...
carried out on 50 brains ...
end so far as the relating of cellular to psychological phenomena goes Thus, speaking of senile plaques, he says,

Manchen andern Autoren beistimmend, konnen wir als unsere Auffassung aussprechen dass diese Gebilde, ob nicht oder ob in grossen Mengen vorhanden, durchaus keine sichere Aussage uber den vorhergehenden psychischen Zustand des Patienten erlauben Similarly Oskar and Cecile Vogt in 1941 compared the minute structure of the thalamus in the brains of four distinguished old scholars who had preserved their intellectual faculties till their death with those of men who had died in senile dementia they concluded that the

involutional process in the thalamus (consisting chiefly of nerve cell degeneration and death in the centro median nucleus) is the same in normal old men with unimpaired intellect as in senile dements. This does not preclude a relation between the normal mental deterioration of old age and the plaques, neurofibrillary thickening and other changes seen in ageing brains but it makes it dubious and certainly does not help in discriminating normal from pathological mental changes.

It has similarly been impossible so far to link the kind and degree of involutional mental changes to either cerebral arterio venous oxygen difference or to the electro encephalogram or to endocrine findings. Pincus and his colleagues at Worcester have given us data about limited stress responses which show no falling off with age in healthy men and I hope Dr Freeman will further illuminate this matter in his paper.

Up to now however purely psychological methods of assessing what is normal have been unchecked by reference to the material basis or accompaniments of the functions in question.

Most of the detailed information about normal psychological ageing has regard to cognition. Intelligence, perception, memory, mental efficiency and speed have been measured and the results indicate a decline, it is true, in intellectual powers but one that is not uniform nor certain nor predictable. On the analogy of physical functions this is what one would expect: allometric growth and allometric decline; in some ripe old age and in others premature senility; no tokens of rapid decay to come, no guarantee against it. There is however very little exact information about how any individual ages mentally: the data we have are derived from analysing the scores obtained by sample populations containing more or less adequate numbers of people of different age groups. This method which has been so fruitful in determining the mental growth of children has led to rather doubtful conclusions when applied to ageing adults. Samples have been small or biased and it has not been safe to assume that

the measuring instrument was measuring the same abilities at different ages

When does decline begin? Earlier work seemed to show that the average scores obtained on intelligence tests diminished at an increasing rate from the third decade onwards But this is now questionable Patrick Slater, on a carefully selected and stratified sample of 2,500 men aged 18 to 42, found no evidence of an increased rate of decline from thirty onwards Vincent, more recently, examined the scores of 7,297 subjects on a verbal intelligence test and found that between the ages of twenty and sixty the mean score declined steadily, showing a linear regression The annual decline of the mean score was found to be likewise constant when other tests had been used it was between ·026 and 030 of the standard deviation of the test Such a finding, though important, is less valuable than one which would establish whether the annual decline in an individual is related to the maximal level attained, say, at sixteen or twenty, or to the rate of increment during the earlier years of life As far as I know there is no evidence at all on these points

A score on an intelligence test is a crude summation If, as seems to be the case, the decline of some abilities is more rapid than that of others, the decrement will be small on some subtests and large on others The abilities least affected have been those concerned with vocabulary and information, while most rapid decline was evident in the ability to deduce relations and adapt to new situations A mean total score would conceal these differences, as well as the differences between individuals The long dispute about a general factor and special factors is relevant to this Balinsky, using Thurstone's method of factor analysis and intercorrelating scores on subtests of the Wechsler Bellevue Scale at different ages concluded that mental organisation changes over a span of years From childhood onwards there is increasing specialisation and then later the various special abilities are integrated into a flexible whole, but with subsequent changes in the relative extent to which special abilities are utilized No

involutional process in the thalamus (consisting chiefly of nerve cell degeneration and death in the centro median nucleus) is the same in normal old men with unimpaired intellect as in senile dements This does not preclude a relation between the normal mental deterioration of old age and the plaques, neurofibrillary thickening and other changes seen in ageing brains but it makes it dubious and certainly does not help in discriminating normal from pathological mental changes

It has similarly been impossible so far to link the kind and degree of involutional mental changes to either cerebral arterio venous oxygen difference or to the electro encephelo gram or to endocrine findings Pincus and his colleagues at Worcester have given us data about limited stress responses which show no falling off with age in healthy men and I hope Dr Freeman will further illuminate this matter in his paper

Up to now however, purely psychological methods of assessing what is normal have been unchecked by reference to the material basis or accompaniments of the functions in question

Most of the detailed information about normal psychological ageing has regard to cognition Intelligence perception memory mental efficiency and speed have been measured and the results indicate a decline it is true in intellectual powers but one that is not uniform nor certain nor predict able On the analogy of physical functions this is what one would expect allometric growth and allometric decline in some ripe old age and in others premature senility no tokens of rapid decay to come no guarantee against it There is however very little exact information about how any individual ages mentally the data we have are derived from analysing the scores obtained by sample populations contain ing more or less adequate numbers of people of different age groups This method which has been so fruitful in determin ing the mental growth of children has led to rather doubtful conclusions when applied to ageing adults Samples have been small or biased and it has not been safe to assume that

prime we cannot tell whether a low score on one or many tests betokens a negligible decrement on an originally low achievement or, on the other hand, a gross falling off from a previously high attainment It is true that we attempt to distinguish between these by devices which assume that some abilities (especially vocabulary) are almost wholly retained while others decline, we therefore detect and measure the decline in terms of this disparity But such devices are, in practice, weak they hardly do justice to the individual differences in constancy or otherwise of vocabulary and information as people grow older, and they cannot tell us at what rate decline has occurred, or will occur in the future Moreover, the decline must be fairly gross to be detected with confidence

It has been urged that the degree of decline in any individual is inversely related to the optimal level of intelligence the more stupid he is, the faster he will become stupider still as he grows old if he is bright he will remain bright These findings bespeak an academic bias It is true that in 45 University professors, aged sixty to eighty, exacting tests showed no unambiguous evidence of decline but perhaps 45 manual labourers aged sixty to eighty who had lived satisfying and healthy lives would also show no unambiguous evidence of decline from their more modest level of intelligence. Moreover in old people so many allowances must be made for sensory and motor defects and unfamiliarity with test procedures that assertions about the more rapid decline of intelligence in the less intelligent must be scrutinized very critically

On the question of deterioration in the highly intelligent, however we can profit by the painstaking labours of Harvey Lehman Limiting himself to the relationship between chronological age and outstanding a[illegible] medicine philosoph[illegible] in all of these the [illegible] late work had occurred before the age of forty, but that there are many instances of exceptional powers retained into the eighth decade of life Verdi, for example, composed Otello at seventy three

matter how the factors are named or interpreted it is evident from Balinsky's data that the same tests measure different abilities in the adult at different ages. Birren likewise by factor analysis of data obtained from the Wechsler-Bellevue Scale administered to subjects between sixty and seventy years of age found that four factors could be extracted from the intercorrelations: they were—verbal comprehension, closure (non-verbal organization of visual perceptual material), memory, and perhaps induction.

The greater scores usually attained on tests requiring verbal comprehension depends mainly on the store of verbal information retained by ageing persons, while ability to acquire fresh information and face fresh situations diminishes.

It seems reasonable that the age of optimum learning and the age of maximum stored information are not coincident and do not correspond to the age of maximum scores on our intelligence tests. *It follows that a scale like the Wechsler-Bellevue needs to be docked of some items and amplified with some others if it is to cover the mental abilities of ageing people and permit comparison of one elderly person with another or with a standard population of the same age.*

It seems clear from the many studies of decline in intelligence in ageing people that a wide range of tests and a series of longitudinal studies of the same individuals are essential if we are to make progress. A wide range of tests is called for to cover the primary mental abilities as fully as possible, and longitudinal studies are needed to allow for the very wide individual differences in optimal performance level and in performance decrement as age advances—individual differences which are submerged and undetectable when the mean scores of large groups of people of different ages are compared. This last difficulty is a most serious one. The great success of the familiar methods of assessing level of intelligence in children of different ages has caused us to concentrate more on performance relative to others of the same age than on rate and kind of change in the individual. So long as we are ignorant of the performance level of an individual in his

and the time when it was learnt We are still some way from being able to measure effective memory, it cannot be taken for granted that "digit span", "sentence repetition", "retention of Turkish English vocabulary" and other such tests, or the recognition of pictures, give a fair estimate of anyone s capacity to retain and reproduce what he has perceived, or tried to learn Moreover performance on most memory tests is influenced by intelligence and habit patterns a comparison of an elderly group with a youthful group can therefore be fallacious Such groups have usually been matched on vocabulary score or socio economic status, neither of which is a wholly safe criterion Even so the more intelligent of the elderly subjects do much better than the rest, and those who have least need to organize their habits in order to learn an intellectual or a motor task show least deficit The learning process, from initial perception to final performance is now being examined with such energy, in relation to a complex theory, that retention in the human subject at different ages will perhaps soon be elucidated, but for the present we cannot dissect the disability that the old have in learning unfamiliar tasks Possibly this is to make a necessity out of a virtue Sir Frederic Bartlett wrote not so long ago that it is insufficient to collect analyse and stu

This of course is what has been done by Welford and his colleagues at Cambridge As Sir Frederic will be speaking later on about this, and about remembering I would only say now that the Cambridge experiments have demonstrated the trouble which the elderly have in organising new incoming data and perhaps also out going action Whether this accounts in part for the greater slowness of the aged in performing many intellectual and motor operations, and for their relative or total failure in speeded tasks is still doubtful Compensatory tendencies may enable a good level of achievement to be maintained in spite of impaired capabilities, so long as the method and

and *Falstaff* at seventy nine Humboldt wrote the five volumes of his *Kosmos* between the ages of seventy six and eighty nine Goethe composed the second part of *Faust* between seventy and eighty Galileo at seventy four, Euler at seventy two Laplace at seventy five were still making valuable original contributions and there are many others It was however, in the continuation of work which had occupied their youth or their prime that these old men were engaged and as Lehman points out, they chiefly assembled knowledge or completed an edifice they had been building all their lives But although qualities of the highest order, such as are essential for great scientific discoveries and the creation of literary and musical works of genius decline with advancing years many of the requisites for distinguished work have clearly been retained It is not yet possible to define or to measure these qualities to which we give loose names such as mental energy or determination But, as Terman has suggested, it is desirable that as full medical and psychological observations as possible should be made of contrasting groups of creative persons including (a) individuals whose productivity declined early and rapidly and (b) others who have remained productive to a much later age Such contrasting groups can be found in the faculty of any large University An important feature of the clinical approach would be the study of motivation in the individual and how these are affected both by specific influences of the immediate environment and by the large social and cultural trends characteristic of the given time and place The effort of the Guilford group at the University of Southern California to develop tests of creative thinking may permit psychometric estimates more relevant to the contrast than existing intelligence tests

Other measurements have been made notably of memory, but these have not added much to the common observation that memory declines with advancing years that individuals differ widely in this respect and that in the same individual the amount of decline varies with the sort of material learned

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timing of the task are under the ageing person's control Many studies have shown, however that the steady process of slowing in simple reactions and choice reactions probably begins in the late teens or early twenties

Personality and awareness of defect may be as important as degree of defect When an elderly man uses a rather different method from that of a younger man to carry out a particular task, because he can no longer command the capacities requisite for the earlier method he may not be aware that he is doing so or much less often his new method may be the outcome of reflection and experiment Personality factors will affect his attitude to disabilities and his success in coping with them But on personality changes in the elderly we have little systematic information Most of it comes from cross sectional studies which cannot tell us whether a trait or constellation of traits is prominent in the elderly only when the same traits have been evident in them when they were young It is for example commonly held that elderly people become more obstinate more self centred more rigid and conservative but the opposite qualities are also observed in many—undue pliancy gossiping, curiosity vacillation and uncritical acceptance these seem less to be the characteristics of age than underlined features of individual personality At present many of the characteristics of old age as ordinarily understood, are attributed to the interplay between the individual and his social environment which moulds his personality as it influences his activities and interests but Kallmann's reports of senescent one-egg twins who have lived their lives far apart suggest that too much weight may be given to social influences in determining personality changes in old age

Rorschach test results have been interpreted as showing in the aged reduced responsiveness to emotional stimuli inability to use inner resources, difficulty in forming satisfactory social relationships and lessened instinctual control, but these findings are by no means regularly encountered Daniel Schuster's old gentleman of one hundred and six vividly illustrates this He provided a rich and productive

(Rorschach) record whose varied content suggests an individual with a multiplicity of interests He is aggressive with a strong drive for achievement, a drive that is accompanied by good creative abilities Emotionally he presents a picture of a basically introverted individual who in emotionally stimulating situations is capable of responding in a warm mature manner he depends upon his own resources Though he may almost appear extraverted in his social contacts (he) actually maintains these on a superficial level for the most part with the exception of a few carefully selected warm associations The interpretation of a Rorschach record is always open to question but in this instance a man who was very old indeed failed to show the changes supposed to be characteristic of the elderly

Other means of inquiry into the personality of the ageing have been interviews attitude questionnaires, and inventories For the most part the populations studied were small and diversely composed The Chicago inquiry, however showed in a large sample of elderly people a lessening of social contacts satisfactions plans and zest The limitations of large scale inquiries about personality are great They are necessarily as a rule concerned with self reported behaviour and with subjective accounts of happiness worries and preoccupations they tell more about what a particular society imposes upon elderly people of a particular socio-economic class and what effect infirmities can have upon conduct, than about lasting change [illegible]

There are [illegible] age of thirty [illegible] often again [illegible] upies that adult personality has a recognisable structure with unchanging components What these components are, and how they can be detected and their mode of organisation classified, is still controversial It is however generally agreed that when certain attitudes and interests are steadily manifested in a consistent way and *occur* often enough with certain other attitudes, we can infer—and by appropriate methods derive—

a single more fundamental factor which manifests itself in them, similarly we measure traits such as perseveration or persistence, which represent an abstraction from several forms of conduct or test response that evidently have a common factor To determine whether personality changes with advancing age and how it does so the surface manifestations of attitude and interest have to be distinguished from the underlying common factors which make up the basic personality As far as I know systematic studies of age changes have been almost entirely concerned with the former no one has applied to the findings the methods of statistical analysis which would reveal whether there are changes in the common underlying characteristics or in the way in which they are organized It is perhaps a vice in this predominantly statistical approach to the problems of personality that it treats the cross section of attitudes and test results at a given moment as a constitutional attribute almost immune from time and change yet there is no inherent reason for this except the great practical difficulty of large scale retesting of the same population or of another population strictly comparable in all relevant respects except age

Many of our assumptions about normal personality changes in older people are influenced by the features which mental disorder wears in them Psychiatrists in particular often allow their experience of paranoid and hypochondriacal trends in senile psychoses to bias their opinion about the normal changes of personality in old age This is understandable since most psychiatrists accept the postulate that mental illnesses not accompanied by constant demonstrable tissue changes—the functional psychoses—express characteristics of personality writ large But do the mental disorders of the elderly in fact exhibit features peculiar to this stage of life?

It is in involutional melancholia that this question has been most obviously disputed backwards and forwards for the last fifty years The characteristics said to be typical of these depressions of later life are hysterical symptoms, hypochondriacal and paranoid ideas, extreme fear and agitation

hallucinations; and catatonic features. Though common, these symptoms cannot be regarded as specific. None of them, and no combination of them, is restricted to the elderly: there is no clinical picture supposedly typical of involutional psychosis that I have not seen in the same form in men and women in their twenties—though it will then be given a different diagnostic label Why agitation, hypochondriacal delusions (especially about the bowels) and paranoid distortion should be so much oftener seen in later life is unclear. Some would relate them to the painful experiences, generating fear and insecurity, which arise out of waning powers, lessened status, and narrowing field of activity as age advances. Others have put forward psychoanalytical concepts of enhanced narcissism, introjection of ambivalently loved and hated objects which are then identified with over-libidinized viscera, and frustration of sexual desires leading to the revival and entrance into consciousness of infantile fantasies; but as these explanations are equally applicable to similar phenomena in younger people, they do not account for the frequency of the constellation of symptoms in the elderly.

Briefly, then, mental illness in middle-aged and elderly people can occur in every form that occurs at other phases of adult life, but is more often characterized by somatic delusions, terror and distrust If the illness is less severe, these characteristics may still be there, but may seem no more than harmless neurotic symptoms They are then taken to be accentuated traits expected in the elderly—"senes morosi, plaintive delirii, suspiciosi' That these traits—suspicion, anxious self concern . . .

is no more tr[illegible]

confidence, of [illegible]

life apart from dementia, are the late realisation of constitutional tendencies, though the genetical factors seem complicated and not specific to senile or involutional illness. The healthier the personality in early life, the less chance of failure to adjust to the changed circumstances and capacities of advancing age

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evident that this has no single source but depends on psycho logical constitution and social and cultural influences Moreover it is not indifferently engaged in compensating for any and every defect but varies greatly in the same individual in respect of different achievements and ways of substitutive attainment To foretell its effectiveness in a senescent we should need to know how it had worked in the past and upon what intact resources it could now draw As Piaget found at the other end of life reorganisation even when unforeseen and sudden never occurs unless the mental combination which determines it has been prepared by earlier experience This analogy with the developmental phase may go deeper adjustment to the problems created by differential increase in abilities may set the pattern that will be followed when the problems are set by differential decline But of course in facing the latter the individual is hampered or helped by the habits

vivid language the predicament of the ageing person who has in a busy career confined his activities to ever renewed tasks given by his environment or who has given up the struggle early in life and depended heavily on personal and occupational supports which are withdrawn when he retires, leaving him empty of purpose and interests

The emphasis in much recent literature about the troubles of the elderly has been *upon* the restrictions set by the

activities With these must be reckoned the social pressures that may have operated over a long preceding stretch of time to unfit *them for the* changes inevitable in later life

For the study of these and of most other mental aspects of ageing investigations prolonged enough to survey the changes in individuals over many years are essential

When progressive dementia and focal symptoms, especially apraxia, aphasia, agnosia, *point to a cerebral degenerative* process of the presenile Pick-Alzheimer type, and this is confirmed post mortem, it is tempting to attribute all the essential symptoms to cellular death in specified parts of the brain; yet the cellular changes found in Alzheimer's Disease have been found also in the brains of undemented old people. The same can be said of senile dementia. The most obvious explanation is that the cellular damage essentially responsible for the dementing process is not disclosed by the histological methods of examination usually employed on the brain. Alternatively, the brain—or the whole organism—may be credited with such great powers of compensating for defect due to local damage that mental abilities may seem intact in *one person while in another with a similarly damaged brain* dementia would be evident. Each of these views is open to objection; they are not, of course, mutually exclusive and may well be complementary. The neuropathologists will no doubt sooner or later tackle the histological problem inherent in the first; as for the second, psychologists have already leant heavily on the concept of compensatory adjustment in explaining individual differences in degree and rate of ageing.

This concept—compensatory adjustment—is still imprecise but indispensable. The falling off in certain abilities, the anxieties about one's material needs, health and survival, the necessity for finding new social roles and preserving social contacts, companionship and affection—these are stresses which can only be met by adaptation calling for fuller use of unimpaired abilities and opportunities to make up for those that are lost. No doubt the ability to compensate needs much *analysis. It is hardly likely that the same ability is* responsible for the reorganisation of effector processes in a skilled task and for the activation of an unsuspected potentiality whereby social misfortunes or bodily decay can be coped with. If the driving force is the need to maintain, in spite of declining or defective ability, a standard of attainment, it is

evident that this has no single source, but depends on psychological constitution, and social and cultural influences. Moreover it is not indifferently engaged in compensating for any and every defect but varies greatly in the same individual in respect of different achievements and ways of substitutive attainment To foretell its effectiveness in a senescent we should need to know how it had worked in the past, and upon what intact resources it could now draw As Piaget found at the other end of life, reorganisation, even when unforeseen and sudden, never occurs unless the mental combination which determines it has been prepared by earlier experience. This analogy with the developmental phase may go deeper. adjustment to the problems created by differential increase in abilities may set the pattern that will be followed when the problems are set by differential decline But of course in facing the latter the individual is hampered, or helped, by the habits

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## DISCUSSION

*Tunbridge* Prof Lewis has emphasized the need to remember that longitudinal studies have an important bearing on the true interpretation of results and that the statistical approach may be quite fallacious Too often, as he said, our standards have been based on a few medical students, or two or three staff members, and the assumption has been made that that is a fair age distribution of population Our standards for all functions, hearing sight, etc, may be completely wrong What we consider at the moment to be normal for seventy may actually be super normal Heredity also may be a factor but it may not be the most important factor How far is longevity in any way chromosome determined? Prof Lewis questioned whether there is such a thing as healthy ageing or pathological ageing I think that is a thesis which [illegible]

. . . is easy when you are dealing with inorganic . . .

nfluence of loss of . . . y were pensioned, . . . passed away very quickly

Another question is, isn't it a fact that the number of ganglion cells diminishes in old people—does that have any correlation with the general mental state?

. . . other changes that . . .

both they found the same changes in the central median nucleus, so that it is impossible to feel confident that there is any necessary connection between intellectual powers and the ganglion or other changes that are observed

. . .

those minimal requirements, which served as struts during their active life are removed on retirement, they go to pieces Then there is a more effective group who remain creative and lead satisfying lives—he gave

Bertrand Russell is one example—because they have inner resources which sustain them irrespective of the jobs which are available or the support that is provided from outside

Going back to your first point, I would mention a distinguished compatriot of Dr Vischer's Dr von Monakow, who wrote an impressive personal review of old age and its favourable aspects when he was himself quite old though still active mentally That essay can be added to the literary accounts you referred to

*Cowdry* May I ask Prof Lewis a question by expressing a view that I have, that it isn't so much the loss of work, though that is very important as it is the change in attitude of mind of older men I believe all of us have lived most of our lives away from the home, and have employed others to some extent—we employ a secretary, we employ 100 men in a factory, we are employers, and have developed the employer complex Now I mean very seriously, when a man is deprived of this work, he goes home he meets there a stronger mentality, and he becomes an employee *I think this is one reason why life expectancy at retirement for men is so low*—not the lack of work, but the tribulations in the turnover from an employer to an employee habit of mind

*Lewis* I think that many of us oscillate between these two rôles even during our active life, don't you?

*Freeman* How does that affect the individual who is a labourer and who is employed both before and after retirement?

*Cowdry* Well of course that is entirely different But I was just

ers in a small or

l suddenly—the

half that of a

woman of sixty five

*Tunbridge* According to a recent study the majority of those dying shortly after retirement showed evidence of considerable physical disability and death was not due merely to cessation of work There was an urge to keep going until retirement which drove them on beyond their physical capacity

*Lansing* Doesn't it follow then that there is a loss of drive at the time of retirement?

*Tunbridge* The goal you see, is achieved at the time of retirement, and is not replaced by another goal

*Lansing* I think that is the point though there is a loss at the time of retirement Apropos of unemployment many have faced it prior to retirement, but it is not disastrous because it is generally agreed to be reversible when one is employable The big blow of retirement is that it is an irreversible process I think that is where the psyche takes a beating

Do I understand, Prof Lewis that you go along with Lehman's concept of peak performance prior to the mid thirties?

*Lewis* I agree absolutely The notable exceptions nearly always arise because of the retained intellectual habits and determination and experience, *rather than because of continuing creative capacity A man* like Euler, for example, can go on solving mathematical problems until

he is eighty, but you don't find people writing a play like *Macbeth* at eighty. There is a difference between the kind of achievement that is possible in later life and really maximal achievement—such as Newton writing his *Principia* before he was forty.

*Lansing*: And I believe it was generally around the age of thirty in most instances.

*Lewis*: Well, it varies for different occupations—chess champions

measuring the dye distribution between the two hemispheres. For example, if we give dye into the right carotid artery and compare the dye content of the two internal jugular veins, we find that in normal younger persons 80 per cent of the dye is on the same side and 20 per cent on the contrary side. Now, when a localizing lesion sets in, this difference between t[illegible]
measure the blood
accurately as possi[illegible]
distribution? There
of workers. The m

[illegible] Prof. Himwich made some observations on this matter—he was concerned with [illegible]

than I do. [illegible] Freeman knows more about this

*Freeman*: We have done some studies in connection with people getting hormones, but I think I could tell you better about it after Betty Rubin has given you the information she has on th[illegible] certainly over a [illegible]
the

Bertrand Russell as one example—because they have inner resources which sustain them irrespective of the jobs which are available or the support that is provided from outside

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... I ... absolutely The notable exceptions nearly always

ageing in an individual in the normal tissue. One can tell it in pathological tissue, but in normal tissue, can you come to any rough conclusions as to the age of an individual?

... between the fibre ...

*Cowdry* ...
the hand? ...
exposure ...
generally isn't exposed, and the anterior surface of the thigh isn't
exposed ...
sweat glan...
atrophy th...

... changes with time in tissue exposed to ...

... often degeneration of elastic fibres may be observed in skin with basal cell carcinoma The degeneration of elastic tissue can only

who collect snails and the longevity of amateur naturalists—I don't offer this as statistically significant—does appear to be quite phenomenal. I was wondering how far we had any direct proof that going on with the job which an old man can do and which leads him to be reasonably respected and keeps him busy and so on, is in fact a means of maintaining general health, because I get the impression that it probably is.

*Lewis* This is commonly assumed and I should think there is a good deal of evidence of the kind that Dr Comfort has brought forward. Karl Pearson of course was much interested in this matter but the difficulty always is that if you take people like snail experts or judges of the High Court or bishops you're dealing with people who have perhaps lived lives immune from many of the stresses which in manual labourers affect their health and therefore their duration of life.

*Comfort* The naturalists are a surprisingly varied group, as a matter of fact, for they are nearly all amateurs; there are few manual labourers but there are one or two among them.

*Freeman* Is it your point, Prof. Lewis, that the more intellectual individual declines at about the same rate as the less intellectual? That is, that the percentage decline may possibly be greater but the actual decline may be about the same?

*Lewis* I don't know, and I don't think the evidence warrants any firm statement.

*Olbrich* Do you notice any difference between the two groups, labourers on one side and intellectual workers on the other side, as to the amount of senile dementia?

*Lewis* There is no evidence that I know of from comparable matched groups.

*Shock* It seems to me that the lack of close correlation between physiological state and mental performance or capacity is probably one of the greatest potential strengths in the entire field of gerontology. The concept that has been put forward that the ageing process sets in and becomes accentuated when the growth process stops has some analogies to mental performance as well. I think we already know some of the things that can continue mental growth in the face of certain of the physiological decrements that often appear with advancing age. Although we do not know as yet how to prevent the physiological changes, we can do something about keeping ageing people mentally active. The low correlation between physiological and psychological measurements, which have plagued us in the past, may be our greatest hope for the future.

## GENERAL DISCUSSION

*Tunbridge* Can we begin with a cellular approach? Is there an age aspect of the cell?

*Aub* This group ought to reach some conclusion about ageing in the cell. Dr Cowdry said there are cytological tests for ageing. There are good pathologists here, and I'd like to know whether they can tell

Cameron No, I haven t, I may have turned a half blind eye. I agree

he specific changes of old age I wonder what they are

Lansing To take further exception to the point that Prof. Cameron makes, many organisms that don t have blood vessels or organized circulatory systems or connective tissue beds to interfere with circulation manifest ageing as we know it in man, mice and other organisms. As Heilbrunn (*Outline of General Physiology*, second ed , Saunders, Phila ) said in his little chapter on ageing, in the last analysis *ageing* is a problem of breakdown of cells Age changes occur in individual cells such as *Paramecium* and in rotifers and other organisms which lack

it o me

pl As

in are

insufficiency

i.e heart, kidney, lung, brain. without regeneration,

2 Organs which are able to regenerate i e epidermis

be found in skin exposed to light ... part in the ... it b ... cess of age ing ... higher differentiation of the epidermis cells The tonofibrils are numerically increased But they are increased in other conditions as well, e.g. in the skin of animals painted with methyl cholanthrene and in precancerous stages

*Tunbridge* Yes, I think we all agree that where you have ... secondary factor—whether th... or what you will you will g... to trauma, is there someth... sidered that he could not ... There is a pattern of secondary degeneration but is there a primary change? I think he even said that the process manifests itself in the same way be it in young or old only the degree changes with chronological age

*Cameron* I think most ... three different chan... found in cardiac mu... sometimes found in other organ cells such as the spleen and kidney That's one ... they claim to be able to ... believe they can but a ... organs for example the ... cells—Max C... ich are shrinking cells showing ... their cytoplasm and nuclear c... All these changes can be induce... case and that is the whole point of my talk I want to know how can you distinguish these from arteriosclerotic changes? You get exactly the same picture in young people with arteriosclerosis for example or various other diseases I frankly can't say, and I don't believe that anyone can say whether these cells are due to ageing or are specific changes

*Rubin* My ... when techniques become delic... chemical nature within the ... centrations of certain enzymes and ... which we can only get at indirectly now in functional studies I think probably you'd be able to find differences in individual cells

*Zub* Have any been found?

*Rubin* None that I know

*Tunbridge* But might your ...

of these ...

The equation of Puetter corresponds to the known sigmoid survival rate in man

There are two different ageing processes one shows a decline from birth to death, the other is characterized by an up and down of development (youth, maturity and senescence)

There is no strict parallelism between the changes of the different

vitality is the broader one, has two aspects, a physical

conducted

*Tunbridge* But you are not wanting the man of sixty to be the man of twenty You are *accepting* the fact that there might be standards of decline? There is not very much difference between you Dr. Miescher is saying he wants 100 per cent vitality of the standard for the age, which I think Prof Verzár would accept as adaptability to the highest *function for* that age

*Miescher* In fact it is simply a question of definition.

In the first group of organs decrease of adaptation begins when there are no more tissue reserves left This occurs normally very late in

of gerontology. We know that arteriosclerosis may start in early childhood and increase during life I think that arteriosclerosis is not a cause of ageing but it becomes intensified by the ageing process because it is a summation effect of very different ætiological factors therefore it must be discussed together with all the problems of ageing In arteriosclerosis it is possible that the tissue reserve diminishes as a consequence of anoxæmia In arteriosclerosis we have to distinguish

local patches in one part of a coronary artery causing local stenosis The consequenc

find the narrow

vitality factor

ing phase is characterized by decreasing resistance capacity of the organism to

is no ageing when

(*a*) $I = R$, that means immortality

(*b*) $I > R$, I and R constants

In a whole population an exponential decline of survivals but no real ageing will occur

$$x = Ae^{-kt} \text{ (or } kt = \ln A - \ln x)$$

e = basis of natural logarithms

t = time

x = number of survivals at time t

A = number of survivals at time O

k = rate of destruction

There is ageing when

(*c*) $I > R$, I constant, R declining (e g following an exponential course)

$$x = Ae^{-kte^{at}} \quad \text{(equation of Puetter, 1921)}$$

a = ageing factor (rate of ageing)

k = destructive factor (rate of destruction)

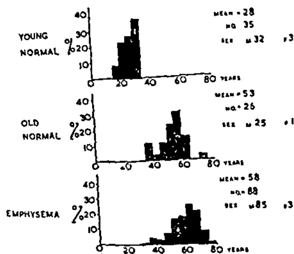



FIG 1 The age distribution of the subjects investigated In this and subsequent similar figures the ordinate is the percentage of the total number of subjects studied

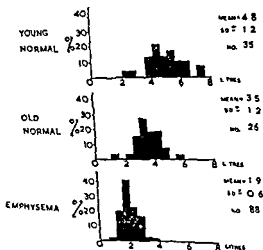



FIG 2 The distribution of the Vital Capacity

# *EFFECTS OF AGEING ON RESPIRATORY FUNCTION IN MAN

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AND R. V. CHRISTIE, M.D., D.Sc., F.R.C.P.,
*Dunn Laboratories, St Bartholomew's Hospital, London*

I HAVE been asked to discuss the effects of ageing on respiratory function. What I propose to do is to describe briefly some of the tests which are now used to assess respiratory function and to contrast the performance of young individuals with the performance of those who have reached middle age. I also propose to bring the disease emphysema into the picture because as far as respiratory function is concerned emphysema is a gross exaggeration of what happens to the lungs with advancing years.

Fig 1 shows the age distribution of the various groups investigated. The sole reason for choosing thirty five years as the dividing line between young and old was that the primary purpose of this investigation was to study the changes which occur in emphysema, a disease which is rarely found before the age of thirty five.

The vital capacity is clearly reduced with age, a fact which has been known for at least half a century. In Fig 2 the "young normals" have a mean vital capacity of 4·8 litres while in the "old normals" the mean is 3·5 litres, a difference which is statistically significant. This change is not due to any decrease in the distensibility of the lung itself, it is almost certainly due to fixation of the thoracic cage, arising from a variety of anatomical changes such as decreased elasticity and immobility of the costal cartilages. In emphysema the vital capacity is still further reduced.

The volume of the lung increases with age (Fig 3). In the younger group this averages 2·9 litres while in the middle-

*Presented by Prof R V Christie

respiratory muscles have to do in order to distend the lungs It is also possible to indicate how much of this work is expended on the elastic resistance of the lungs and how much on the viscous or non elastic resistance (McIlroy, Marshall and Christie, 1954)

In Fig 4 is shown a summary of our results In eight healthy individuals below the age of thirty five, between 70

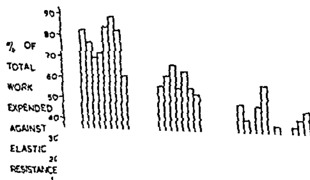



FIG 4 The proportion of respiratory work expended against the elastic resistance of the lung

and 80 per cent of the work of breathing was expended against elastic resistance In seven healthy individuals over the age of fifty, only between 50 and 60 per cent was expended against elastic resistance, and in emphysema the percentage is even lower *It is fair to conclude that with advancing years the elastic resistance or elastic recoil of the lungs is reduced* (McIlroy and Christie, 1954)

So much for the mechanical aspects of lung function

*The sole function of the lung* is to ventilate the blood and if [illegible]
least t [illegible]
lated [illegible]
[illegible], in other words each alveolus should

aged it averages 3 5 litres and in emphysema the average rises to 4 7 litres One reason for this increase, and possibly the sole reason, is that the elastic recoil of the lung diminishes with age During life the lungs remain distended because the elastic recoil is counterbalanced by traction of the chest wall and diaphragm, and expansion of the lung can be brought

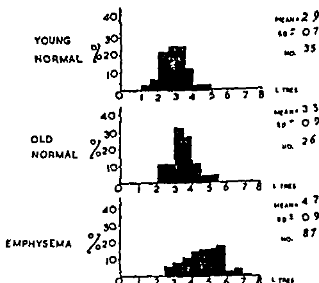


FIG 3 The distribution of the Functional Residual Capacity (i e the volume of air in the lungs at the end of an ordinary quiet expiration)

about either by increased traction, such as occurs on inspiration, or by diminution in elastic recoil That the elasticity of the lung does in fact diminish with age can be shown by direct measurement, and I will just say a word about the technique we have used On inspiration the intrapleural or intrathoracic pressure becomes more negative, and this fall in pressure is a direct measure of the force exerted on the lungs by the muscles of inspiration The tidal air is a measure of the volume change which occurs If these two, the intrapleural pressure and the tidal air, are measured simultaneously it is quite easy to calculate the amount of work the

respiratory muscles have to do in order to distend the lungs It is also possible to indicate how much of this work is expended on the elastic resistance of the lungs and how much on the viscous or non elastic resistance (McIlroy, Marshall and Christie, 1954)

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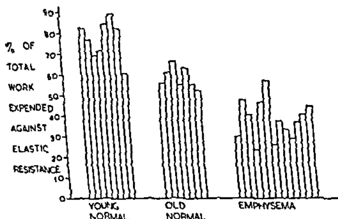



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... , in other words each alveolus should

get just its fair share of each breath that is taken. Secondly, the air in each alveolus *should be fully exposed to the lung capillaries*, in other words, the alveolar air should come into proper contact with the pulmonary blood. Both of these functions can be measured, and I will first say a word about the efficiency of ventilation which, surprisingly enough, can be measured quite easily and accurately.

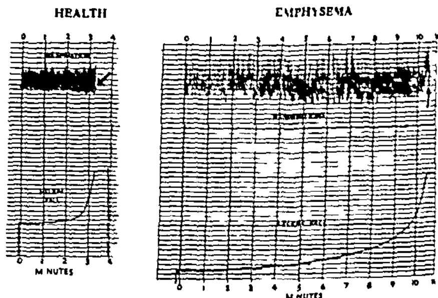



Fig. 5. The efficiency of gas mixing in the lungs measured by the helium dilution method. In a healthy young individual (on left) equilibrium between spirometer and the lungs is reached after 30 breaths; in a patient with emphysema (on right) the equilibrium is only reached after [illegible] breaths.

If an inert and relatively insoluble gas such as helium is breathed it will mix with the other gases in the alveoli but practically none will be absorbed (Bates and Christie, 1950).

In Fig. 5 is shown the record of a young normal individual who starts to breathe from a spirometer containing 13.5 per cent helium. The helium percentage, which is measured electrically by a katherometer, falls, and after two minutes

or 30 breaths equilibrium is established between lung and spirometer, with a helium percentage of 9 12 If the lung had been perfectly efficient from the point of view of a ventilation engineer—i e if all the alveoli had been equally ventilated—it can be calculated that equilibrium should have been reached in 23 or 24 breaths whereas in fact 30 breaths were required and it is true that most normal individuals

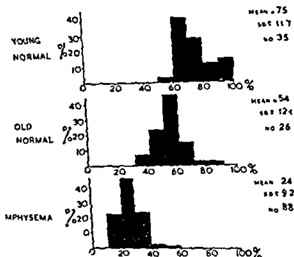



Fig. 6 The efficiency of gas mixing in the lung expressed as a percentage of perfect efficiency is shown on the abscissa

operate at 70 or 80 per cent efficiency as far as ventilation is concerned

The record of a patient with emphysema is also shown in Fig 5 and here the story is a very different one Mixing was only complete after ten minutes or 150 breaths

In Fig 6 is shown a summary of our results Young normals operate at an average of 75 per cent efficiency while the figure for the older group is only 54 per cent a difference which is statistically significant This can only mean that with advancing years ventilation of the lungs becomes more uneven, and this is presumably a sequel of loss of elasticity

Lastly, is there any change in the ability of the inspired air to make proper contact with the pulmonary blood? It might seem that the obvious way to settle this point would be to analyse the arterial blood and find out just what has happened to the blood as it passed through the lungs. To do this with any accuracy it is not enough to measure the $O_2$ saturation of the arterial blood; both the alveolar and arterial

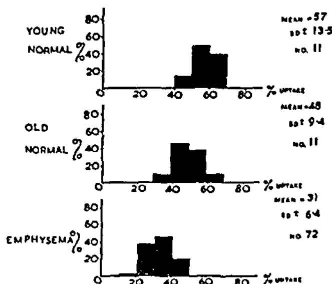



FIG. 7. The percentage of CO removed from the inspired air when breathing 0.1 per cent CO in air is shown on the abscissa.

$O_2$ tensions have to be measured, the technique of which is difficult and laborious. More straightforward is the use of carbon monoxide. As you all know, *hæmoglobin has a great affinity for carbon monoxide*. In fact if a healthy individual breathes a low concentration of CO most of the gas which reaches the alveoli will be taken up by the blood (Bates, 1952). The expired air will therefore contain very much less CO than was inspired. If however some of the inspired air were wasted in ventilating avascular areas a very different result might be expected. In these bloodless areas the CO would not be absorbed and therefore the concentration of CO in the expired

air would be increased This is exactly what happens with increasing age (Fig 7)

In young normals the average CO uptake is 57 per cent while in the older age group it is down to 48 per cent and in emphysema it falls to 31 per cent With advancing years the contact between alveolar air and pulmonary blood or diffusing capacity as it is sometimes called becomes impaired

In conclusion as age increases the chest wall becomes less mobile and the elastic recoil of the lung is reduced In consequence the volume of the lung increases and the efficiency with which gases are mixed in the alveoli is reduced The capacity of the lung to ventilate the pulmonary blood is also slightly impaired All of these changes occur in a greatly exaggerated form in pulmonary emphysema

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(A full bibliography will be found in these references).


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McILROY M B MARSHALL R and CHRISTIE R V (1954) *Clin Sci*, 13 127


## DISCUSSION

*Franklin* I wonder if Prof Christie will tell us what he thinks is the real connection of ageing with emphysema

*Christie* I think it has some relationship to what Medawar said yesterday You remember he said that senescence is due to a combination of inborn deterioration or change in vulnerability and deterioration due to repeated injury or stress and strain I think th s [illegible]
appl es [illegible]

this which is respo[illegible] are in the same dir[illegible] develop in old age [illegible] *one of Dr Vischer's* [illegible] emphysema, and P[illegible] had emphysema We have calculated from our results that if a healthy individual were to live on indefinitely and the changes in his lungs [illegible]ved, he would develop emphysema

[illegible] Milton Landowne and Mr Arthur Norris, our laboratory has been collecting observations on age changes in pulmonary function We have also used a helium wash out technique for estimation of the various lung compartments (Norris, Landowne and Shock, 1952, *Fed Proc*, 11, 114) The study is still in progress, but I [illegible]

[illegible] was found in estimates of maximum voluntary ventilatory capacity [illegible]

[illegible] 2 0 l at age eighty to ninety The inspiratory reserve volume also fell from about 3 5 l to 1 8 l over the same age range The age decrement in expiratory reserve volume was not nearly as great—1 1 l at age [illegible]

[illegible] showed [illegible] *reduction* [illegible] ty) *After* [illegible] city In contrast, the vital capacity continued to decrease up to the age of eighty I think that in general the results we obtained are in agreement with the cases Dr Christie has reported

We have not done carbon monoxide measurements and I'd like to ask Dr Christie how one can separate the effects of the exchange of carbon monoxide from any changes that might occur in cardiac output with age

*Christie* All this work has been done in conjunction with Comroe in [illegible] the air with the pulmonary blood Our work on patients with mitral stenosis shows that they do not get the same reduction of CO uptake although the cardiac output is decreased

*McCance*: This has been to me a delightfully simple exposition of a [illegible]

[illegible]

experimentally in rats

I also want to ask how far the decrease of lung volume which you found in old cases is a functional one? Have you tried changes by adrenaline or ephedrine sprays in these cases? That might show that a part of it is functional and is caused by a decrease of skeletal muscle tone, and not by elasticity

[illegible]

make these measurements wh[illegible]
that is really the only approac[illegible]
no effect on older individuals [illegible]

[illegible]

*Tunbridge*: You indicated that there may be a change, rather more marked, at another age period [illegible]
tion, or is t[illegible]

*Christie*: [illegible]
are not suff[illegible]
after the [illegible]

[illegible]

[illegible] now through the l[illegible]
that the rate decreases [illegible]

problem, it really is a cardiovascular one rather than a respiratory one, although of course the two are very close to each other when you come to the pulmonary capillaries. I don't think such work has been applied to ageing.

*Franklin*: Could you elaborate on the mixing of air in the lungs? I ask because in some (anaesthetized) animals there is a very uneven distribution of air in the lungs. In the rabbit, for instance, you get a very patchy distribution, and in the cat to a lesser extent. In the human subject it is even, according to your evidence, isn't it?

*Christie*: Unfortunately you have mentioned two animals I know nothing about. In dogs it is even. In human beings it is 80 per cent [illegible]

*Christie*: I think the sex distribution was indicated on the slides. Most of the individuals we studied were men. It is a very curious thing and there is no clue as to why it should be, that emphysema occurs almost solely in men. A woman can have chronic bronchitis or asthma and yet she seems to be much more immune to this stress and strain effect than man. But our figures are mainly on men, and therefore I can't really say anything about the sex distribution.

*Franklin*: Prof. Verzár, some years ago you showed the effects of lowered oxygen partial pressure, which produced a change very similar to one of ageing, namely, a greater resting degree of expansion of the lungs. Could you correlate that with what we have heard this morning?

*Verzár*: We believe that there is a third form of respiration regulation besides the depth and the rhythm of inspiration, and that is the increase of respiratory surface by an increase of lung volume. One observes it immediately if one starts physical work, or if the oxygen pressure in the inspired air is decreased. One can demonstrate it by body plethysmography. The meaning of the reaction is an increase of respiratory surface by better ventilation. This reaction might decrease with old age by a decrease of muscle tone, and perhaps by a decrease of elasticity of the lung.

*Christie*: Well, there are many complex facets to this, but I think the [illegible]

*Tunbridge*: Is there any evidence from the work that Pugh did on the Everest expedition that the Sherpas have a similar ability?

*Christie*: I don't know.

# CHANGES WITH AGE IN DIFFUSION COEFFICIENTS OF SOLUTES FOR HUMAN TISSUE MEMBRANES

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*Division of Gerontology Washington University St Louis Mo*

The determination of diffusion coefficients of solutes for preparations of tissue membranes constitutes a valuable method of measuring the permeability of the tissue In the field of gerontolgy this technique may be applied to the investigation of the effect of age on the permeability In the present communication the results of diffusion coefficient measurements on samples of the human aorta and human tentorium cerebelli are reported

## Experimental

Samples of the thoracic aorta and tentorium cerebelli were obtained at the St Louis City Morgue Sterile instruments were employed for the removal of the tissues The adventitia was stripped off the aorta after which the intima (with attached subintimal tissue) was separated from the media A preparation of each layer was then inserted in a sterile diffusion apparatus The tentorium cerebelli was employed directly for diffusion studies

The diffusion coefficient measurements were carried out under sterile conditions by the procedure of Kirk and Johnsen (1951) This method permits determination of diffusion coefficients of both gaseous and non gaseous solutes under a constant total pressure

The details of the assembled diffusion apparatus are shown in Fig 1 The apparatus consists of two 50 ml glass syringes the bottoms of the barrels have been removed and the barrel

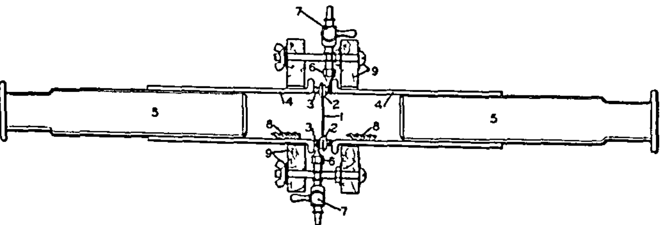



Fig 1 1 Membrane 2 Metal diaphragm with bevelled circular opening 3 Hard rubber 1¼ inch slip joint washer 4 Syringe barrel 5 Syringe plunger 6 Gauge No 12 needle with tip cut off at right angle 7 One way metal stop cock with two male outlets 8 Screw shaped glass piece to provide stirring of solution 9 Wooden clamp with brass screws

tops ground plane The membrane is interposed between the ends of the syringes and is held in place by two metal dia phragms and two hard rubber rings A needle is inserted through each rubber ring the outer ends of the needles are closed by insertion of a one way metal stopcock

The gas or non gaseous compound to be investigated is dissolved in buffer medium heated to 37° C and the solution introduced into one of the compartments of the apparatus The other compartment contains plain buffer medium After both compartments have been filled with solution the diffu

allowed to elapse for establishment of temperature equilibrium and for initial penetration of the gas or compound through the membrane before withdrawal of samples for analysis

The technique provides for the withdrawal of samples from the diffusion apparatus at any time during an experiment In studies on gas diffusion samples of the solution are trans ferred without contact with the air to the extraction chamber of a Van Slyke apparatus for gas analysis If conditions of analysis permit samples should be withdrawn in immediate succession from the two compartments of the diffusion apparatus at the beginning and end of a diffusion period This will be possible in the case of diffusion studies on non gaseous solutes and in investigations on gas diffusion provided two Van Slyke apparatuses are available

The diffusion coefficient is defined as the units of the sub stance diffusing through 1 cm ² of the membrane in one minute at a concentration gradient of 1 unit per ml per cm (Hill 1928 29) For experiments in which samples for analysis are withdrawn in immediate success on from the two com

on

$$(c_3 - c_4) = (c_1 - c_2)e^{k\frac{A}{L}\left(\frac{1}{V} + \frac{1}{V_1}\right)}$$

where,

$\lambda$=diffusion coefficient,

$c_1$=concentration of solute on donor side at beginning of period,

$c_2$=concentration of solute on recipient side at beginning of period,

$c_3$=concentration of solute on donor side at end of period,

$c_4$=concentration of solute on recipient side at end of period,

A=area of membrane in cm $^2$,

L=thickness of membrane in cm ,

$V_1$ and $V_2$=volumes of solution in the two compartments expressed in ml , *and*

$t$=time in minutes

## Results

### Aortic tissue

Determinations have been made of the diffusion coefficients of nitrogen, oxygen, carbon dioxide, lactate, iodide and glucose in experiments on 51 samples of intima preparations and 50 samples of media preparations The age of the individuals from whom the samples were obtained ranged between ten and eighty years

The mean diffusion coefficients observed for these preparations are shown in Table I The table further contains the calculated values for the products diffusion coefficient $\times \sqrt{MW} \times 10^5$ It will be seen from the table that a fair agreement was found between the product values for nitrogen, oxygen and carbon dioxide, and that the product values for these gases were over two times greater than the product values for lactate and glucose These findings might indicate the presence in the aortic membrane of a set of smaller pores (permitting the passage of compounds of MW 28 to 44) and a set of larger pores (permitting the passage also of compounds of MW up to 180)

### Table I

Average Diffusion Coefficients and Mean Values for Products D x $\sqrt{MW}$ x $10^5$

| Solute | MW | $\sqrt{MW}$ | Intima (with attached subintimal tissue) Diffusion coefficient | Diffusion coefficient x $\sqrt{MW}$ x $10^5$ | Media Diffusion coefficient | Diffusion coefficient x $\sqrt{MW}$ x $10^5$ |
|---|---|---|---|---|---|---|
| Nitrogen | 28 | 5 29 | 0.000469 | 248 | 0 000552 | 291 |
| Oxygen | 32 | 5 65 | 0 000502 | 283 | 0 000579 | 327 |
| Carbon dioxide | 44 | 6 63 | 0 000404 | 267 | 0 000375 | 248 |
| Lactate | 90 | 9 49 | 0 000123 | 116 | 0.000084 | 80 |
| Iodide | 131* | 11 45 | 0 000318 | 365 | 0 000258 | 296 |
| Glucose | 180 | 13 42 | 0 000104 | 139 | 0.000076 | 102 |

* Radioactive isotope

### Table II

Mean Diffusion Coefficients ( x $10^5$) Observed for Various Age Groups

| Age group (Years) | Number of samples | Nitrogen | Oxygen | Carbon dioxide | Lactate | Iodide | Glucose |
|---|---|---|---|---|---|---|---|
| Intima (with attached subintimal tissue) | | | | | | | |
| 10 - 39 | 13 | 39 3 | 43 9 | 35 9 | 9 3 | 25 3 | 7 4 |
| 40 - 59 | 20 | 50 0 | 55 0 | 40 0 | 9 9 | 31 5 | 10.1 |
| 60 - 80 | 16 | 49 2 | 50 1 | 44 2 | 16 6 | 36 3 | 12 8 |
| Media | | | | | | | |
| 10 - 39 | 13 | 49 9 | 50 5 | 32 9 | 6 . | 23 0 | 6 1 |
| 40 59 | 20 | 54 9 | 60 7 | 30 7 | 7 6 | 24 | 7 3 |
| 60 80 | 17 | 57 7 | 60 5 | 41 9 | 10 7 | 29 9 | 9 1 |

where,

$\lambda$=diffusion coefficient,

$c_1$=concentration of solute on donor side at beginning of period,

$c_2$=concentration of solute on recipient side at beginning of period,

$c_3$=concentration of solute on donor side at end of period,

$c_4$=concentration of solute on recipient side at end of period,

A=area of membrane in cm.$^2$,

L=thickness of membrane in cm.,

$V_1$ and $V_2$=volumes of solution in the two compartments expressed in ml., and

$t$=time in minutes.

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## Table I

Average Diffusion Coefficients and Mean Values for Products D x $\sqrt{MW}$ x $10^5$

| | | | Intima (with attached subintimal tissue) | | Media | |
|---|---|---|---|---|---|---|
| Solute | MW | $\sqrt{MW}$ | Diffusion coefficient | Diffusion coefficient x $\sqrt{MW}$ x $10^5$ | Diffusion coefficient | Diffusion coefficient x $\sqrt{MW}$ x $10^5$ |
| Nitrogen | 28 | 5 29 | 0 000469 | 248 | 0 000551 | 291 |
| Oxygen | 32 | 5 65 | 0 000502 | 283 | 0 000579 | 327 |
| Carbon dioxide | 44 | 6 63 | 0 000404 | 267 | 0 000375 | 248 |
| Lactate | 90 | 9 49 | 0 000123 | 116 | 0 000084 | 80 |
| Iodide | 131* | 11 45 | 0 000318 | 365 | 0 000258 | 296 |
| Glucose | 180 | 13 42 | 0 000104 | 139 | 0 000076 | 102 |

* Radioactive isotope

## Table II

Mean Diffusion Coefficients ( x $10^5$) Observed in Various Age Groups

| Age group (Years) | Number of samples | Nitrogen | Oxygen | Carbon dioxide | Lactate | Iodide | Glucose |
|---|---|---|---|---|---|---|---|
| Intima (with attached subintimal tissue) | | | | | | | |
| 20 39 | 13 | 37 3 | 43.9 | 35 9 | 9 8 | 25 3 | 7 4 |
| 40 59 | 20 | 50 0 | 55 0 | 40 0 | 9 5 | 31 5 | 10 1 |
| 60 80 | 18 | 43 2 | 50 2 | 44 2 | 16 6 | 36 3 | 12 8 |
| Media | | | | | | | |
| 20 39 | 13 | 47 9 | 50 5 | 32 9 | 6 4 | 23 0 | 6.2 |
| 59 | 20 | 54 8 | 60 7 | [illegible] | 7 6 | 24 | 7 5 |
| 80 | 17 | 57 7 | 60 5 | 43 9 | 13 7 | 29 9 | 9 1 |

The average diffusion coefficient values observed for the age groups ten to thirty-nine, forty to fifty-nine, and sixty to eighty years are shown in Table II. It will be seen from the table that a definite tendency was found for the diffusion coefficients to increase with the age of the individuals. The significance of this tendency is also evident from the calculated coefficients of correlation between age and the diffusion coefficients (Table III).

Table III

Correlation Coefficients : Age / Diffusion Coefficients for Intima and Media Preparations of the Human Aorta

| | Intima | | Media | |
|---|---|---|---|---|
| | r | t | r | t |
| Age / Nitrogen | + 0,28 | 2.04 | + 0.35 | 2.60 |
| Age / Oxygen | + 0.18 | 1,27 | + 0.29 | 2.08 |
| Age / Carbon dioxide | + 0.23 | 1,66 | + 0,36 | 2.67 |
| Age / Lactate | + 0.46 | 3.60 | + 0.41 | 3.12 |
| Age / Iodide | + 0.38 | 2,89 | + 0.39 | 2.93 |
| Age / Glucose | + 0.58 | 4.99 | + 0.44 | 3.35 |

In connection with the data reported in Table III it should be noted that the total lipid and cholesterol contents of the aortic membranes were likewise found to increase with advancing age. The coefficient of correlation age/total lipid was +0.18 for the intima samples and +0.32 for the media samples. The corresponding r values for the correlation age/cholesterol were +0.40 and +0.31.

## Tentorium cerebelli

Diffusion studies with this connective tissue membrane of comparatively simple structure were carried out on 35 samples derived from individuals ranging in age between one and

seventy eight years Only the diffusion coefficients of carbon dioxide and glucose were estimated

The mean diffusion coefficient values for the age group one to thirty nine, forty to fifty nine, and sixty to seventy-eight years are shown in Table IV In contrast to the findings in the experiments on the aortic samples the data fail to show any consistent change in permeability of the cerebellar tentorium with age

Table IV

Mean Diffusion Coefficients ( x 10[5]) Observed for Various Age Groups for Human Tentorium Cerebelli

| Age Group ( Years ) | Number of samples | Carbon dioxide | Glucose |
|---|---|---|---|
| 1 - 39 | 8 | 43.6 | 12.9 |
| 40 - 59 | 17 | 44.2 | 11.4 |
| 60 - 78 | 10 | 44.9 | 12.6 |

## Summary

Diffusion experiments were carried out with nitrogen, oxygen, carbon dioxide, lactate, *iodide* and glucose on preparations of the intima and the media of the human thoracic aorta, *using the diffusion procedure* of Kirk and Johnsen (1951) The diffusion coefficient values showed a def[illegible]

[illegible] the findings in the experiments on aortic tissue, diffusion studies on *preparations of the human* tentorium cerebelli failed to show any increase in *permeability* of that tissue with age

The investigation was supported by a research grant (PHS-891) from the National Heart Institute of the National Institutes of Health, Public Health Service

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## DISCUSSION

*McCance* To what extent do you consider these tissues are alive? I could not see any part of that apparatus designed to keep those cells aerated, and we all know now from the work which is going on at a cellular level, that if you do not keep even a single cell oxygenated its metabolism is completely disorganized, it swells up, changes its size, [illegible]

*Kirk* I did not go into all the details of the experiments because of the limitation of time In the diffusion studies which I reported at least one of the two compartments of the apparatus contained oxygen It has been demonstrated in a previous study (*J Gerontol*, 9, 10 1954) that human aortic tissue after removal from the body will continue to show respiration for periods of up to several weeks when kept under sterile conditions The $Q_{O_2}$ values of such tissue preparations can be measured by means of a technique which we developed recently (*J. biol Chem* 199 675 1952 208, 17, 1954) This technique is considerably more sensitive than the ordinary Warburg procedure, since it permits determination of $Q_{O_2}$ values as low as 0 01 We have shown that under ordi[illegible]

[illegible] requires because, physiologically it is bathed as a rule, in a medium containing so much Am I right in thinking that you separate the muscular tissue from the intima before incubating the latter?

*Kirk* No In the media we have some smooth muscle cells but the values I gave for the $Q_{O_2}$ refer to the aortic wall And there is not much difference in the $Q_{O_2}$ of the human intima and media The maximum value is about 0 30

*Franklin* Have you done any experiments on animals? Because I think it might answer Dr McCance's question if you took some of these pieces and used them to repair blood vessels in other animals

*Kirk* Well I have carried out determinations of the rate of respiration and glycolysis of the dog aorta (*J Gerontol*, 9 10 1954) The studies showed that the $Q_{O_2}$ of the dog aorta is approximately twice that of the human aorta about 0 65 0 70 is the maximum $Q_{O_2}$ found in dogs Our observations on the respiration of aortic tissue stored at refrigerator temperature between tests are in essential agreement with the data by Pierce and his associates (*Ann Surg*, 129, 333 1949) who showed that

it was possible in many instances to obtain growth of fibroblasts in tissue cultures from segments of aortas stored for periods of thirty to fifty days at 4° C in a salt solution-serum medium

*Franklin* You haven't used a piece of the old vessel for repair?

*Kirk* No, I have done no transplantation studies.

*McCance* I think there is more in it than whether the tissue is alive sufficiently to use for "repair" purposes If, for instance, you take a piece of kidney and cut thin slices of it and immerse it in the solution

content of the wa
of oxygen Since
to the square roo

dioxide will be 23 8 × $\sqrt{32}/\sqrt{44}$, or 20 3 times greater than that of oxygen (at the same partial pressure difference)

In the field of chemistry, however, especially when dealing with diffusion kinetics, Hill's definition of the diffusion coefficient is generally employed, and as mentioned in the presentation of my data—it was this coefficient which was used in the present study According to Hill's def [illegible] for car [illegible] to $\sqrt{}$ [illegible] m samples presented today the average diffusion coefficients observed for carbon dioxide and oxygen were 0 000404 and 0 000602 respectively. This gives a ratio between the coefficients of 0 805, a value which is in fair agreement with the ratio of 0 853 obtained for the diffusion of the gases in water

*Lansing* I wonder if one can properly compare the intima of the young [illegible]

very thick elastica interna In this stripping the elastica interna goes with the endothelium The elastica interna has periodic fenestrations which are quite gross and rather widely separated so that between the fenestrations there are very definite barriers to diffusion Now, if one examines the separated intima of the old, it's a very different story One finds the endothelium, a very rich bed of fibroblasts, collagenous fibres, ground substances and so on which may measure as much as a millimetre or two, depending upon the pathological state of the tissue and then, far beneath all this material, one finds the fragmented and disrupted elastica interna which is so badly damaged that often it does not go along with the sub endothelial material in stripping Thus in the young there may be a mechanical barrier to diffusion in the elastica interna which is lacking in the old This alone may contribute to your data The implication would be that the sub endothelial material between the elastica interna and endothelium is not a barrier to diffusion

*Medawar* Dr Kirk, if the membranes you are working with are deliberately killed by non precipitants or narcotized, do you still get the age differences? And you mentioned using oxygen uptake as a criterion of the viability of the cells are there age differences in the oxygen uptakes of these various membranes?

*Kirk* I have not made any studies on the effect of enzyme inhibitors or various poisons on the membrane permeability And as far as the oxygen consumption is concerned our data (*J Gerontol* 9 10 1954) failed to show any change with age in the oxygen consumption of the aortic tissue membranes

*Bean* Do you believe that the increase in cholesterol is a cause an effect, or an independent parallel of the change in permeability you found in relation to arteriosclerosis?

*Kirk* I have no real opinion on that point We merely carried out determinations of the total lipid and cholesterol content of the aortic membranes to have a quantitative index of the pathological changes in

the tissue, but I have no opinion on the direct relation between the cholesterol content and the diffusion coefficients

*Bean* Was there an individual correlation as well as an average correlation?

*Kirk* We have only carried out experiments on 50 membranes, and the number of samples for each five year group is too small in my opinion

# THE CHANGING INCIDENCE OF CERTAIN VASCULAR LESIONS OF THE SKIN WITH AGEING

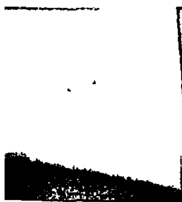
W. B. BEAN, M.D.,

*Department of Medicine and the University Hospitals of the College of Medicine, State University of Iowa, Iowa City, U.S.A.*

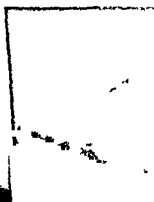
THE subdued tones of discussion of the sombre aspects of ageing have echoed down the immemorial lanes of history, and the subject has been treated variously by writers and philosophers from Cicero to Ponce de Leon, from Seneca to Browning. Our recent preoccupation with gerontology in an ageing society casts oblique Darwinian light on those who, growing old, pontificate upon growing old. Gray hair, obesity, *wrinkles, presbyopia, loss of muscle tone, the menopause,* the burden of disease in later decades—all these have had increasing attention. Yesterday's introductory probings aiming for a definition of ageing without a prior definition of life or of time, Newtonian or Einsteinian—illustrate the large difficulties of our problem. Is ageing merely the running down of a wound up clock, a Calvinistic view, or is it such a process in a clock which is buffeted about and thus does not run as long as its unbuffeted control?

Yesterday's suggestion that we get more data by observing *the process and progress of ageing as it goes*, rather than look at the artificially stopped frame in a moving picture of panoramic dimensions, gives a mere clinical observer some excuse for participating in this colloquium which I take to mean talking to each other. Thus as junior in wisdom and in years but still a member of an ageing society, I join in our increasing and self conscious focus upon gerontology. I hope I may avoid a geriatric hazard not always escaped in the

(1)



(c)



(3)

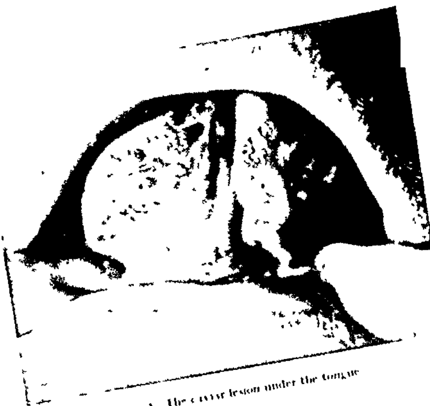


Fig. 4 The caviar lesion under the tongue

academic arena that of becoming mellow before I have grown ripe

My brief discussion today concerns vascular lesions in the skin and their increasing incidence with advancing years For the most part it emphasizes observation rather than interpretation I have no evidence to demonstrate preponderant environmental or genetic influences The study represents merely the first step in clinical research, which is systematic observation and recording of data I have only vague notions of why the several types of lesions occur at all and can give you only speculation about the effect of increasing age upon them

The observations began about fifteen years ago when I failed to get any help about the nature of arterial spiders from the Nestors of American medicine at Johns Hopkins or Harvard I was sufficiently curious to attack the problem from the simple approach of the natural historian Having become interested in one kind of vascular alteration of the skin I noticed others closely or distantly related, or sometimes merely confused in the medical writings on the skin The data reported here are based on a study of about 700 persons with arterial spiders and palmar erythema, 75 persons with Osler s disease and more than 1,000 patients surveyed in detail for cherry angiomas, venous stars and caviar lesions The venous lake on the ear has been studied systematically only recently

## Spider Nævi

To try to throw light on the significance of the eruptive spider nævus (Fig 1) of liver disease and pregnancy I made a series of studies which led to the hypothesis that such vascular lesions were overgrown and hypertrophic end arteries whose increase in size was probably caused by œstrogenic hormones In chronic disease of the liver these hormones are in high concentration in the blood because the liver cannot adequately inactivate, destroy or excrete them In pregnancy the liver functions well but the level is high because the hormones

are produced in great quantity Since approximately 10 *per cent of normal persons may have one or more vascular* spiders their clinical significance is important only when there are many, when new ones appear or when they enlarge *Age* The eruptive spider nævi occur in the childbearing period in women and in the cirrhotic patients mostly in the thirties and forties There is no apparent relation to age *per se* They occur in very young or very old persons

Palmar erythema as an acquired vascular change in the skin has the same backgound, course and age incidence as spider nævi The relationship to hyperœstrinæmia is not so close though there is the same clinical background

## Venous Stars

As the name implies the venous star (Fig 1) is a stellate system of collecting veins visible in the skin and larger than those in normal skin *They are small varicose veins or venules* appearing usually upstream in venous collecting systems of the skin where pressure is regularly or intermittently high They are common on the lower legs and thighs in association with larger varices *Blood flows from the* periphery to the centre thence to an underlying collecting vein Such lesions may appear in large numbers over the chest in persons with obstruction of the superior vena cava during the period of development of collaterals *Age* Fig 2 shows that as age increases many more persons are found to have venous stars There is a consistent tendency for more women than men to be affected in any age group This is associated with the well known tendency for women to have more and worse varicose veins than men

## *Cherry Angiomas*

There are many names for the cluster of small dilated capillary vessels which form the characteristic bright red ruby spot, De Morgan's spot senile angioma or capillary angioma *The size and shape vary but the typical spot is*

round the small ones flat, the large ones elevated and dome-shaped In most persons when the large ones occur, magnification of twenty times reveals many smaller ones with but one

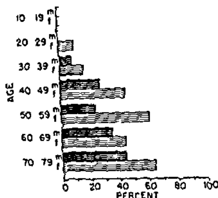



FIG 2 The increasing incidence of venous stars with increasing age In every decade women outnumber men The bars indicate the percentage of all subjects found to have one or more lesions.

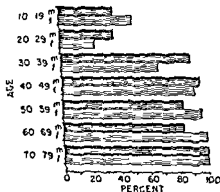



FIG 3 Incidence of cherry angioma by sex and decade

or a few capillary tufts *Pressure* partly blanches the lesion and the colour and the natural shape are restored after about a minute *Age* Fig 3 shows the rapidly rising incidence

with increasing years so that in the thirties about three quarters of the persons observed have the lesions and they are almost always found in the aged. Not only do more people become affected but the spots are more numerous and larger.

## Caviar Lesions

The caviar lesion is a small, roughly spherical or dome shaped varicose enlargement of the poorly supported vein

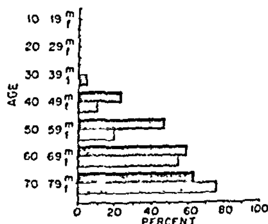



Fig. 5. Incidence of caviar tongue lesions by sex and decade

in the collecting system under the tongue. The small ones develop as an outpouching of a branch of a communicating vein near the ranine veins. Pressure with a glass slide causes them to empty. Knots and clusters of such vessels with a dark blue or black colour resemble caviar or buck shot (see Fig. 4). Anatomically the caviar lesion is a dilated vein with hypoplastic endothelium but a fairly thick wall. There are no inflammatory changes. *Age.* There is a sharp and fairly steady increase in incidence with increasing age and the lesions grow larger and more numerous (Fig. 5).

## Osler's Disease

### (*Hereditary Hæmorrhagic Telangiectasia*)

The cutaneous lesion in Osler's disease is usually flat, punctiform with sharp margins Solid bluish lesions may occur The mucous surfaces of the body, especially of the mouth and lips are favourite sites Bleeding is common as would be expected from the vascular fault, a deficiency of smooth muscle and elastic tissue in small veins, arteries and capillaries The trait is transmitted without sex linkage as a Mendelian dominant Recently a number of persons with this disorder have been observed to develop pulmonary arteriovenous aneurysms *Age* I have observed some children with no skin lesions who bled from the nose and after the age of puberty atypical telangiectases appeared in the skin Bleeding is rare before puberty It most often becomes a clinical problem during the third decade and so remains throughout life There may be slow increase in size of some lesions others may vanish but the change is usually gradual over a period of years

## Venous Lakes

In recent years I have made observations on a dilated venous structure found on the ears and lips of old persons They are rare in women but not so rare in old men In colour they are dark blue P
plete blanching Th
reveals a thin vascul

## Discussion

The life of man witnesses many changes in blood vessels—the great proliferations during embryonic life, the sharp shifts of the neonatal period the tendency for birthmarks and hæmangiomas generally to disappear during the early years of life But there are vascular alterations of another class readily observed in vessels of the skin which increase in size,

with increasing years so that in the thirties about three quarters of the persons observed have the lesions and they are almost always found in the aged. Not only do more people become affected but the spots are more numerous and larger.

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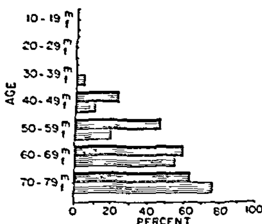



Fig 5 Incidence of caviar tongue lesions by sex and decade

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puzzle it might be said with more justification than is now possible that

> "small inferior veins
> From me receive that natural competency
> Whereby they live"
>
> *Coriolanus, Act I, Scene I.*

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number and in frequency of occurrence with ageing In Fig 6 the effect of ageing is noticed in the somewhat reciprocal relation of persons exhibiting all three of the venous lesions and those who have none While the cherry angioma is proliferative and grows larger as time goes on the vessel walls are thin and atrophic Caviar lesions are dilated and varicose veins of small size Their enlargement may be favoured by increases in pressure especially because they are not protected

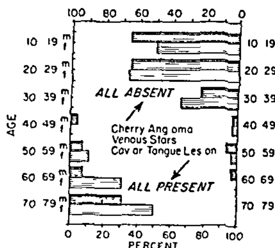



Fig 6 Age and sex distribution of persons with all three venous lesions and those with none

by supporting tissue without or valves within Venous stars increase with ageing chiefly in relation to chronic stasis in large veins, but may occur whenever the venous pressure remains high

These observations provide a starting point for detailed work on the increase in frequency, in size, and in number with ageing Nothing emerges to tell whether they are the result of the intrinsic process of ageing, the mere passage of time, or whether they result from the stress and strain inherent in life and increase with ageing because environmental stimuli accrue as time passes Should further work help solve the

elastic tissue of the media and the relations of such elastic tissue changes to atheromata "The only feature that is clear is that the less definite a lamina (elastic) protecting the media, the more definite is the atherosclerosis" Ophuls observed that "the most important change caused by ageing of the arterial wall is a gradual diffuse distension due to the progressive deterioration of the elastic tissue" Wells argued strongly for the importance of elastic tissue failure in arteriosclerosis The fact is that a large volume of data had been accumulated describing a characteristic fraying and fragmentation of elastic tissue in arteriosclerosis frequent reduplication of the elastica interna and development of a profound affinity by elastic tissue for calcium salts Curiously enough Anitschkow who fathered experimental cholesterol atherosclerosis pointed out that lesions of the arterial wall coupled with intimal fibrosis predispose to atheromata in the presence of hypercholesterolæmia

It is quite understandable that Anitschkow's production of atheromata by the administration of cholesterol resulted in an intensive effort over the last twenty years to link arteriosclerosis to faulty lipid metabolism These studies have been strongly reinforced by the lipoprotein studies of Gofman and of Barr by the dietary studies of Keys and by many others too numerous to enumerate As these studies developed it became increasingly difficult to relate elastic tissue changes in the arterial wall to the ætiology of arteriosclerosis

Our laboratory has been led to the conclusion that arteriosclerosis may be considered to be not one but two diseases One disease involves a defect in cholesterol metabolism or circulation while the second disease is manifested by a breakdown in the structure of the elastic elements in the media of arteries accompanied by a calcification of this elastic material This point of view was supported by our observations on human material that the elastic tissue breakdown occurs prior to the formation of atheromata that the changes in elastic tissue are associated with age and may occur without atheromata (the converse is not true) and that

# AGEING OF ELASTIC TISSUE AND THE SYSTEMIC EFFECTS OF ELASTASE

ALBERT I. LANSING, Ph.D.
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It is interesting to note that although collagenic and elastic fibres have closely related origins, they are remarkably different functionally, chemically, and anatomically. The collagenic fibre is a tough, stretch-resistant element that is quite rich in hydroxyproline and is characterized by regular transverse periodicity. The elastic fibre, as the name indicates, is very elastic, contains only a trace of hydroxyproline and is annoyingly free of structural characteristics. The latter is especially true of elastic fibre preparations in the electron microscope. *A propos* of the present discussion it may also be added that these two fibre types differ in their susceptibility to pathological alterations, there are a great number of diseases affecting collagen and the generalized fibrosis of ageing is almost a diagnostic feature of that process. On the other hand there are but a limited number of diseases of elastic tissue, and the ageing of this material is highly localized. Hass (1939) has reviewed much of the data concerning elastic tissue diseases.

In my present discussion it is intended to review some of the data on age changes in arterial elastic tissue and its relation to human arteriosclerosis, and to note that the age changes in dermal elastic tissue of the human are strikingly different from those found in arteries.

*Arteriosclerosis*, edited by Cowdry in 1933, reveals a surprising unanimity of opinion in regard to the nature of this disease. Fox, in outlining the phylogenetic aspects of arteriosclerosis, emphasized the significance of changes in the

elastic tissue of the media and the relations of such elastic tissue changes to atheromata 'The only feature that is clear is that the less definite a lamina (elastic) protecting the media, the more definite is the atherosclerosis" ( Ophuls observed that 'the most important change caused by ageing of the arterial wall is a gradual diffuse distension due to the progressive deterioration of the elastic tissue". Wells argued strongly for the importance of elastic tissue failure in arteriosclerosis The fact is that a large volume of data had been accumulated describing a characteristic fraying and fragmentation of elastic tissue in arteriosclerosis, frequent reduplication of the elastica interna, and development of a profound affinity by elastic tissue for calcium salts Curiously enough, Anitschkow, who fathered experimental cholesterol atherosclerosis, pointed out that lesions of the arterial wall coupled with intimal fibrosis predispose to atheromata in the presence of hypercholesterolæmia

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Two dimensional paper chromatography appears to be an effective means of characterizing elastin After complete hydrolysis, tryptophane exists as a trace in elastin (Lansing et al 1951) but is a significant component of other tissue proteins By tracing the disappearance of the ninhydrin colorized tryptophane spot in the chromatogram one can readily determine when contaminants of elastin are removed This was done in the NaOH digestion series and it was determined that the tryptophane spot disappeared after forty to fifty minutes At this time, glycine, proline, leucine, isoleucine and valine were readily identified in the chromatogram and minor spots for aspartic and glutamic acid were present The finding of these amino acids as the principal components of elastin is consistent with published analytical data for the amino acid composition of elastin (Neuman and Logan 1950)

There was a progressive reduction in the amount of residual material through forty to forty five minutes of digestion, after which time the residual dry weight was constant through sixty minutes These data seem to indicate that digestion of elastic tissue in hot dilute alkali for less than forty five minutes is not adequate to remove extraneous materials from the tissue (Fig 1)

Microscopic examination of samples digested in NaOH for time periods less than forty five minutes revealed variable amounts of collagen and muscle after staining with Mallory's *procedure* At and after forty five minutes no collagen or muscle could be demonstrated and the elastin was optically homogenous refractile, and apparently free from contaminating structures Likewise, elastin prepared from ligamentum nuchae was free of collagen in so far as could be determined by light microscopy and electron microscopy It was birefringent when stretched, or dried, had a refractive index of 1 534 was resistant to digestion by crystalline trypsin (Armour) and stained effectively with orcein, resorcin-fuchsin, or the Verhoeff procedure

when these two lesions co exist the accumulation of cholesterol in the intima occurs after the elastic tissue of the underlying media has broken down

Whether or not this formal hypothesis is sound is not too important. The cardinal point is that in human arteriosclerosis there is an almost invariable association of medial elastic tissue degeneration with the conspicuous atheromata The questions are what relation is there between cholesterol accumulation in the intima and elastic tissue breakdown in the media; and, is there a common factor responsible for the production of both of these lesions?

It would be excessively repetitious to present once again all of the histological and analytical data which have contributed to the development of the point of view of my laboratory. For the purposes of this discussion it might be more appropriate to make a few points on the chemistry of elastic tissue in relation to age and arteriosclerosis and to attempt to evaluate factors that condition elastic tissue in these two states Since all of our data depend upon the method of preparation of elastin this procedure will be described before making three points, first, that although elasticity of arteries decreases with age, there is no apparent loss of elastin, second, that elastin extractable from old human aortas has an amino acid composition distinct from that of young elastic tissue, and third, that elastic tissue degeneration as measured by calcification thereof occurs as a function of age

## Preparation of elastic tissue

Elastin may be prepared in a reproducible form and with several objective measures of purity A slight modification of the method of Lowry, Gilligan and Katersky (1941) was applied to the tunica media of fresh aortas The tissue was refluxed in methanol or ethanol for one hour and in acetone for a second hour. This defatted material was then digested at 98° C in 0 1 N NaOH and small samples taken off at five minute time intervals for chromatographic, histological, and chemical *analyses.*

content is significant Certainly there is no significant gain or loss of elastin after the third decade of life

The data for the pulmonary arteries followed a somewhat different pattern from those of the aortas During the first three decades of life the media of the pulmonary artery

1
[illegible] lmonar[illegible]
years [illegible]
[illegible] solved [illegible]

contained slightly less than 31 per cent elastin This increased slowly but steadily throughout life to 34 per cent in the seventh decade and to almost 37 per cent in the eighth decade The increase with age of elastin although small is statistically significant

It would appear then that at least after the second decade of life the elastin content of elastic vessels such as the aorta and pulmonary artery either remains constant or actually

**Constancy with age of human arterial elastin**

A total of 110 human aortas and 106 human pulmonary arteries from which both intima and adventitia had been removed by stripping were analysed for elastin content by the method just outlined.

The results obtained by these procedures on aortas ranging in age from stillborn to one hundred and three years are

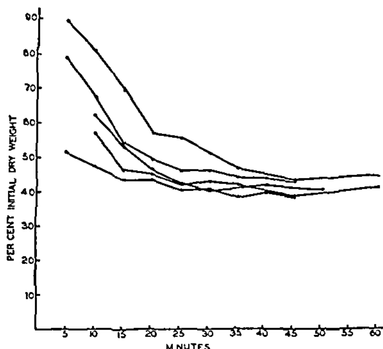



FIG. 1. Hydrolysis of human aorta in 0.1 N NaOH showing that incubation at 98° C. for at least 45 minutes is needed to procure a constant residuum of insoluble elastin.

summarized in Fig. 2. The average elastin content of the media during the first two decades of life is slightly over 48 per cent while the average elastin content in the third decade and thereafter drops to values ranging between 41.1 and 44.1 per cent. Because of the limited number of specimens available for analysis in the first two decades it is difficult to decide whether or not this apparent drop in medial elastin

most of that from old individuals settled to the bottom A sample of the suitably washed light fraction (young light) was prepared from several young aortas and some of the heavy material (old heavy) was prepared from old aortas Both of these materials were analysed for calcium and the contents of 18 amino acids

**Table I**

PERCENTAGE OF PRINCIPAL AMINO ACIDS IN PURIFIED ELASTIN FROM AORTA

| | *Young* | *Senile* |
|---|---|---|
| Aspartic | 0 14 | 1 5* |
| Glutamic | 1 8 | 4 4* |
| Glycine | 26 5 | 23 0 |
| Valine | 16 4 | 14 3 |
| Proline | 13 6 | 13 3 |
| Leucine | 6 8 | 7 4 |
| Isoleucine | 3 5 | 3 7 |

* Significant increase

In Table II are shown the contents of 18 amino acids in the *light fraction* of young aortic elastin and the heavy fraction of old aortic elastin prepared by suspension in sucrose in the manner described previously In the case of the 7 amino acids which were determined on whole elastin (*Table I*), the differences observed between the young light and old heavy fractions were all similar to those found for the young and old whole elastin The old heavy elastin showed increases [illegible]

[illegible] groups in the old heavy sample In addition there was a decrease in the content of alanine All of the other amino acids showed increases of varying degree in the old heavy elastin Approximately 90 per cent of the nitrogen of both samples was accounted for by the analyses The light fraction contained 1 14 per cent calcium while the heavy fraction contained 6 39 per cent

increases The loss of arterial elasticity with age cannot be attributed to a loss of elastic tissue It is more likely due to a change in the properties of elastic tissue with age

## Amino acid composition of young and old arterial elastin

Microbiological assay of aortic and pulmonary elastin prepared in the usual manner was effected by the method of Roberts, Ramasarma and Lewis (1950) Nitrogen analyses indicated a content between 15 and 16 per cent suggesting that elastin is largely protein (Highly significant increases in the contents of aspartic and glutamic acids were noted in the samples of old aortic elastin as compared to those of the young elastin) in confirmation of the chromatographic findings There were also increases in the contents of amide nitrogen However, there was a four fold increase in the excess of dicarboxylic acids over amide nitrogen, showing that the quantity of free carboxyl groups was increased significantly in the older specimens The mean values for valine, proline, and glycine contents were somewhat lower in the older samples, while the leucine and isoleucine contents in the two groups were closely similar (In contrast to the findings for aortic elastin the pulmonary elastin did not show increases in aspartic and glutamic acid contents with age) The other amino acids also did not change significantly The contents of the proline, leucine, isoleucine, and valine were virtually identical with those found in young aortic elastin However, the contents of glycine and the dicarboxylic amino acids were higher in the pulmonary elastin The quantity of free carboxyl groups was at least twice as great as that found in young aortic elastin, while the range showed some overlap with that of the old aortic elastin It was found that a separation of fractions having differing specific gravities could be achieved by differential centrifugation of finely ground elastin (Wiley mill) in sucrose solution (sp gr 1 30) Most of the material in samples of aortic elastin from very young individuals floated on the solution after centrifugation, while

## Age calcification of medial elastic tissue

The question has repeatedly arisen whether the breakdown and calcification of medial elastic tissue is a sequel to atheromatosis or whether this is an age dependent phenomenon In an attempt to resolve this question atheroma free segments of the upper abdominal portions of human aortas were collected and both adventitia and intima were separated by stripping To guard against chemical contamination of the

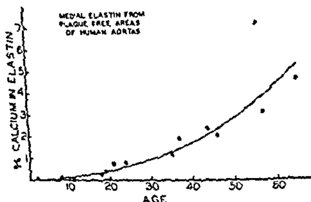



Fig 3 Analyses of the calcium content of elastin from human aortas using grossly normal specimens. *In plaque-free areas there is a progressive increase with age in the calcium content of the elastin*

selected areas by adjacent and overlying atheromata or necrotic and calcified plaques we selected for analysis 14 aortas which possessed an over all minimum of atheromatosis Little difficulty was experienced in collecting atheroma-free tissue in the young group while considerable selection was required in the older specimens

The separated tissues collected in this age series were analysed for calcium content by the method of Salomon, Gabrio and Smith, and for cholesterol content by the method of Kingsley and Schaffert The data as summarized in Fig 3 show that there is no significant age change in the amount of

**Table II**

AMINO ACID COMPOSITION OF YOUNG LIGHT AND OLD HEAVY ELASTIN

| *Amino acid* | *Young light** *g N per 100 g N* | *Old heavy†* *g N per 100 g N* |
|---|---|---|
| Aspartic | 0.38 | 1.11 |
| Glutamic | 1.83 | 3.01 |
| Glycine | 26.10 | 21.30 |
| Proline | 10.10 | 9.[illegible]0 |
| Leucine | 4.5[illegible] | 4.7[illegible] |
| Isoleucine | 2.10 | 2.33 |
| Valine | 13.00 | 11.50 |
| Alanine | 23.18 | 21.58 |
| Lysine | 0.49 | 1.17 |
| Arginine | 1.78 | 4.3[illegible] |
| Histidine | 0.15 | 0.75 |
| *Cystine* | *0.06* | *0.10* |
| Methionine | 0.06 | 0.3[illegible] |
| Phenylalanine | 1.71 | 1.97 |
| Tyrosine | 1.45 | 1.76 |
| Tryptophan | 0.06 | 0.[illegible]4 |
| Serine | 0.29 | 0.70 |
| Threonine | 0.65 | 1.13 |
| Amide N | 2.90 | 2.86 |
| Total | 90.83 | 90.1[illegible] |

*This preparation contained 1.14 per cent calcium and 14.9 per cent N
†This preparation contained 6.39 per cent Ca and 12.5 per cent N

These data indicate that the amino acid composition of young and old arterial elastin is significantly different.

It is doubtful that the shift in amino acid distribution represents a change with time in the amino acid composition of a pure protein. The elastin studied may be a mixture of two or more proteins and the changes observed may be related to differences in the ratios of the separate constituents. How such a change can come about is not yet known. It is possible that the proteins laid down later in life may be different from those formed earlier because they are formed under the influence of fibroblasts which themselves have undergone age changes.

a matrix material which together make up the elastic fibre

In a recent study of the phylogenetic distribution of elastase, Lansing, Rosenthal and Alex (1953) not only found this enzyme in a teleost fish but also determined that it was present in the islet rather than acinar tissue *Lophius piscatorius* (goose monk, angler fish) like several other teleosts has islet tissue that is anatomically separate from the acinar portion of the pancreas Each, then, can be collected separately and in relatively pure form We found the elastase activity to be confined exclusively to the islet tissue and were thus led to suspect that elastase has a systemic rather than digestive function This suspicion is supported by the well known observation that elastic fibres resist digestion in the alimentary tract, adult human pancreatic juice has failed to show elastase activity the yield of elastase from whole pancreas is extremely low and beef pancreas is an excellent source of elastase Since this animal is herbivorous there would appear to be no need for a digestive enzyme for elastic tissue

Balo and Banga have recently indicated (1953) that the elastase content of the pancreas of human arteriosclerotics is substantially less than normal Close inspection of their tabular data shows that age is as much a variable in their material as is arteriosclerosis For example, the group of arteriosclerotics which yield a mean of 9 elastase units per gram had an average age of sixty-one years, the group of non arteriosclerotics who came to autopsy because of other diseases had an average age of thirty four years and a mean of 155 elastase units per gram, lastly, the group of non arteriosclerotics who came to autopsy through violent death had an average age of thirty-one years and 208 elastase units per gram The possibility appears to exist that loss of elastase may be related to arteriosclerosis and/or ageing

Although elastase has been crystallized by a rather laborious procedure with a very low yield this material is generally prepared as little more than a pancreatic extract by acid

medial elastin or in the calcium and cholesterol contents of the intima There is a small increase in the cholesterol content of the media and a thirty fold increase in the calcium content of the medial elastin It would appear from these data that the development of an affinity for calcium by arterial elastic tissue is an age conditioned process

It seems clear that change in composition of human arterial elastic tissue, which is almost invariably associated with overlying atheromatosis is age conditioned That age alone is not effective is attested to by the fact that the pulmonary artery is normally resistant to elastic tissue change at all ages The pulmonary artery does develop typical elastic tissue changes as well as atheromatosis when it is subjected to stress as in pulmonary hypertension

In presenting these several forms of data I have not been attempting to propose that the primary lesion of arteriosclerosis is elastic tissue breakdown rather than accumulation of lipid in the intima My point is that arteriosclerosis is a complex disease, at least in the human arteriosclerosis is a product of both accumulation of lipid in the intima and degeneration of elastic tissue in the media of arteries The question is Is there a common basis to both of these lesions or is their occurrence coincidental? I suspect that the former possibility is valid and that the material called elastase may be implicated in both elastic tissue and lipid metabolism The data are far from complete and elastase is as yet poorly defined but sufficient experimental data are available to warrant serious consideration of the role of this material in arteriosclerosis

Elastase exclusively extractable from the pancreas, was originally prepared and characterized as an enzyme by Balo and Banga (1950) It is apparently specific for elastic tissue and acts upon the substrate to convert a fibrous protein to the globular form in solubilizing elastic tissue there is no apparent release of peptides or amino acids In this laboratory elastase has been used to work out the fine structure of elastic fibres The enzyme solubilizes both elastic fibrils and

arterial elastic tissue, the data are largely histochemical As a matter of fact there is some question in my mind as to whether or not the elastic tissue changes in senile elastosis . In so far as can be . in the dermis of skin . ments, such as face,

Table III

UPTAKE OF [14]C LABELLED ACETATE IN NORMAL RABBIT AORTIC ELASTIN AND IN ELASTIN FROM THE AORTAS OF ELASTASE TREATED RABBITS

| Animal Number | Exp | Dry wgt AORTA (mg) | Dry wgt ELASTIN (mg) | % ELASTIN | Activity cts/min | S.A cts/min/100 mg |
|---|---|---|---|---|---|---|
| 121 | 2 day | 102 | 43 | 42.2 | 6.9 | 15.8 |
| 122 | cont. | 119 | 48 | 40.3 | 4.2 | 12.7 |
| 123 | | 94 | 34 | 36.7 | 8.4 | 24.3 |
| 124 | | 224 | 45 | 20.0 | 6.6 | 14.2 |
| 125 | | 135 | 52 | 38.6 | 13.7 | 26.7 |
| | | | | 39.3 | | 18.7 |
| 126 | | 145 | 60 | 41.1 | 6.5 | 10.8 |
| 127 | 2 day | 116 | 50 | 43.1 | 5.4 | 10.6 |
| 128 | Elastase | 133 | 56 | 42.1 | 5.7 | 10.2 |
| 129 | | 109 | 41 | 37.7 | 8.4 | 20.5 |
| 130 | | 119 | 41 | 40.0 | 7.3 | 14.4 |
| | | | | 40.9 | | 12.8 |
| 21 | | 94 | 32 | 34.0 | 361.5 | 1064 |
| 22 | 7 day | 105 | 39 | 37.1 | 96.0 | 255 |
| 23 | cont. | 151 | 67 | 44.3 | 59.2 | 134 |
| 24 | | 112 | 43 | 38.4 | 26.6 | 71.3 |
| 25 | | 105 | 36 | 33.3 | | |
| | | | | 37.4 | | 380.5 |
| 26 | | 149 | 53 | 37.3 | 30.5 | 81.7 |
| 27 | 7 day | 125 | 45 | 36.2 | 24.0 | 47.1 |
| 28 | Elastase | 126 | 54 | 42.9 | 16.6 | 34.5 |
| 29 | | 142 | 51 | 35.9 | 14.5 | 40.3 |
| 30 | | 135 | 48 | 40.0 | 12.2 | 25.0 |
| | | | | 39.0 | | 46.7 |

back of the neck, and hands, it does not occur in skin that is normally covered by clothing It would seem that this elastic tissue lesion is a product of external stresses operating for long periods of time

In senile elastosis the normal bed of delicate elastic fibres in the pars papillaris of the dermis is replaced by a coarse mat of fibres 3 5 times thicker in diameter than normal In severe lesions this mat may extend proximally to include the pars reticularis. These elastic fibres have several properties

extraction and salting In such extracts the elastase activity *in vitro* or *in vivo* is destroyed by boiling The elastase activity of pancreatic extracts persists after fat extraction and dialysis Curiously enough although these conditions would suggest that elastase is a protein, the systemic effects to be outlined were obtained by oral administration of the material A unit dose per rabbit was that amount of elastase extractable from 5 grams of pancreatin (Viokase, Viobin Corp , or Wilson s concentrated pancreatin)

In two groups of tracer studies using $^{14}C$ labelled acetate (COOH) and glycine ($CH_3$) with and without elastase, it appears that the oral administration of this material has significant effects on the metabolism of aortic elastic tissue Rabbits, in groups of five, were given 0 1 mc of either of the two isotopes by intraperitoneal injection and followed for two to twenty eight days Animals were sacrificed at various time intervals after injection of the glycine or acetate, the aorta from the arch to the diaphragm was removed, fat extracted, reduced to elastin, hydrolyzed and transferred to planchets for counting The only difference between the controls and experimental rabbits was that the latter received daily doses of elastase for the duration of the experiment The data are summarized in tabular and graphic form (Table III & Fig 4) The results with the controls indicate that the metabolism of elastic tissue is considerably like that of collagen as established by Neuberger using comparable doses of the isotopes Every indication is that the turnover of elastic tissue is very low, and indeed after administration of elastase is even lower than in the control It is difficult to propose a formal explanation for this result, one possibility is that elastase removes a particular component from elastic tissue, one that is substantially labelled in the control Whatever the final explanation for this phenomenon may be, it is clear that oral administration of elastase has an *in vivo* effect on the isotopic labelling of elastic tissue

The information we have on ageing in elastic tissue in human skin is much less complete than that available for

arterial elastic tissue; the data are largely histochemical. As a matter of fact there is some question in my mind as to whether or not the elastic tissue changes in senile elastosis should be referred to as an age change. In so far as can be determined senile elastosis occurs only in the dermis of skin that is chronically exposed to the elements, such as face,

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| 123 | | 95 | 34 | 36 7 | 8 4 | 24 3 |
| 124 | | 116 | 45 | 38 8 | 6 6 | 14 2 |
| 125 | | 135 | 52 | 38 6 | 13 7 | 26 7 |
| | | | | 39 3 | | 18 7 |
| 126 | | 146 | 60 | 41 1 | 6 5 | 10 8 |
| 127 | 2 day | 116 | 50 | 43 1 | 5.4 | 10 8 |
| 128 | Elastase | 131 | 56 | 42.8 | 5 7 | 10 2 |
| 129 | | 109 | 41 | 37 7 | 8 4 | 20 5 |
| 130 | | 120 | 41 | 40 8 | 7 3 | 16 6 |
| | | | | 40 9 | | 13 8 |
| 21 | | 96 | 32 | 34.0 | 361 6 | 1064 |
| 22 | 7 day | 105 | 39 | 37 1 | 94 0 | 253 |
| 23 | cont | 151 | 47 | 44 3 | 59 2 | 134 |
| 24 | | 112 | 43 | 38 4 | 26.6 | 77 3 |
| 25 | | 102 | 36 | 35.3 | ---- | - - - |
| | | | | 37 8 | | 380 5 |
| 26 | | 169 | 63 | 37 3 | 30 5 | 81 7 |
| 27 | 7 day | 126 | 48 | 38 2 | 18 0 | 47 1 |
| 28 | Elastase | 126 | 54 | 42.9 | 14 8 | 34 3 |
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| 123 | | 94 | 34 | 36 7 | 8 4 | 24 9 |
| 124 | | 116 | 45 | 38 8 | 6 4 | 14 2 |
| 125 | | 135 | 52 | 38 6 | 13 7 | 26 7 |
| | | | | 39 3 | | 18 7 |
| 126 | 2 day Elastase | 146 | 60 | 41 1 | 6 5 | 10 8 |
| 127 | | 116 | 50 | 43 1 | 5 4 | 10 8 |
| 128 | | 131 | 56 | 42.8 | 5 7 | 10 2 |
| 129 | | 109 | 42 | 37 7 | 8 4 | 20 5 |
| 130 | | 110 | 41 | 40 0 | 7 3 | 26 6 |
| | | | | 40.9 | | 13 8 |
| 21 | 7 day cont. | 94 | 32 | 34 0 | 361 6 | 1064 |
| 22 | | 105 | 39 | 37 1 | 94 0 | 253 |
| 23 | | 151 | 57 | 44 3 | 59 2 | 134 |
| 24 | | 112 | 43 | 38 4 | 26.6 | 73 2 |
| 25 | | 102 | 36 | 35 3 | | |
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| 26 | 7 day Elastase | 169 | 63 | 37 3 | 30 5 | 81 7 |
| 27 | | 126 | 48 | 38 2 | 18 9 | 47 7 |
| 28 | | 128 | 54 | 42 9 | 14 8 | 34 5 |
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| 30 | | 135 | 65 | 40 8 | 12.2 | 29 8 |
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which distinguish them from the elastic elements found in senile arteries

The senile elastic tissue of arteries manifests a weak acidophilia whereas the opposite is true in senile elastosis When the elastic fibres of the latter lesion are stained with a dye such as methylene blue or toluidine blue the fibres exhibit

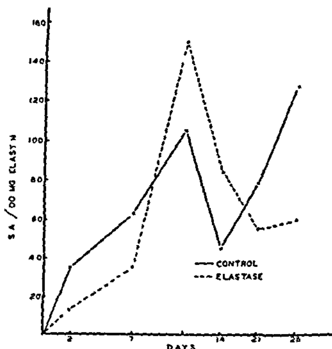



FIG 1 Graphs showing the uptake of $^{14}C$ labelled glycine in normal rabbit aortic elastin and in aortic elastin of rabbits treated with elastase

a strong basophilia No evidence of metachromasia has been observed so far by us Another striking difference between ageing of arterial and dermal elastic tissue exists in the calcium content of the two As has already been indicated the ageing of arterial elastic tissue may be measured in terms of the amount of calcium it contains Juvenile arterial elastic tissue is free of calcium, with age it progressively takes up calcium, as much as 15 per cent of the dry weight of the

purified elastin In micro incinerated preparations the calcium in elastic fibres may be recognized as very heavy deposits of a brilliant white ash when the specimen is viewed with dark field We have made micro-incinerated sections of a number of specimens of skin exhibiting senile elastosis to date we have found no evidence of a tendency toward mineralization of the elastic fibres in this lesion

There has been some question as to the nature of the fibres found in senile elastosis all of the evidence found in our laboratory would establish that the fibres are elastic They stain typically with the usual stains for elastic tissue resorcin fuchsin orcein and the Verhoeff reagent Thin sections of osmic acid fixed fibres of normal skin and of senile elastosis are indistinguishable both are quite free of distinctive architecture Lastly incubation of small cubes of fresh skin exhibiting senile elastosis with elastase shows typical solubilization of the elastic elements therein The digested areas are negative after Mallory or elastic tissue staining It would appear that we are dealing with a bizarre type of elastic tissue

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## DISCUSSION

McC ee I was fascinated by Dr Lansing s communication because the possible effect of age on the chemical structure of proteins has

ageing placenta

*Tunbridge* I am sorry Dr. Hall is not able to be here, because he has

much of the non protein material present, since it destroys mucopolysaccharides It will also, however, cause hydrolysis of peptide linkages in the main protein chains themselves and therefore it is surely not the best reagent for the preparation of a pure undegraded sample of "elastin" Using methods which, though still chemically drastic, are less likely to produce the effects of alkalis, we do find marked differences First of all the polysaccharide is retained in quite high concentration We do not agree with Balo and Banga that the amount of carbohydrate is of the order of 10 per cent, because if you look at their methods, they have not allowed for the amino acid content of their hydrolysates, which would probably be included in any determinations of reducing

perties lies elsewhere, probably in the fact that their polysaccharide content differs, both qualitatively and quantitatively The difference in physical properties would suggest that elastin has a rubber like constitution with enormous stretch right up to the point of breaking as opposed to collagen which only stretches slightly before breaking It is difficult to get pure specimens, and one hesitates to draw conclusions, but I do think Hall's work shows that there is a definite difference between the two proteins It is not clearcut, the chemical differences when a more gentle method of separation is used are not as great as those usually suggested It is fascinating, and it is in line with what McCance has said

*Aub* I liked Dr Lansing's presentation, but there is one question that puzzles me, and that is the extraordinary increase in calcium in this elastin If I'm correct in my chemistry, calcium phosphate would still be intact in 1/10 N sodium hydroxide You only spoke of calcium and not phosphate, but in your last slide I saw that phosphate also went up in your preparation I wonder whether the calcium is really attached to

[illegible] and I can say that the calcium exists largely as an

*Tab* But you get such a large percentage of calcium—a tremendous change *Is that all attached to your elastin? Could some of it be* phosphate or apatite?

*Lansing* I think it is calcium phosphate And, as I say, some of it at least is directly linked to the elastin molecule How far the change goes in crystallization I can t say Anatomically in micro incineration all the *mineral is directly in or on the elastic fibres Visual observation, of* course, doesn't establish chemical continuity, but anatomically all the calcium salts are in or on the elastic fibres when viewed in dark field micro-incinerations

*Medawar* I m interested in the idea raised by Lansing that the elastic fibre systems of the skin and of the blood vessels may have *very different* properties Dr Bean mentioned that in Osler s disease there was im-

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gross scale—a square foot of skin at a time—and obtained slightly *higher results than those we have quoted earlier* But whichever way you look at it, be it by weight, volume or crude analysis, it would seem that at the most there is not more than 5 per cent, and probably less elastin in dermal tissue

blood vessels are less well supported and they are very vulnerable to trauma The extraordinary thing is that it increases with age, it is present in about 2–3 per cent of the population at the age of sixty, and *in 25 per cent of all we have been able to examine at ninety*, there is a linear relationship With it goes scarring, tendency to hæmorrhage and so on, but it is definitely altered collagen Dr von Albertini would agree that it is a totally different collagen degradation product from that which you get in the rheumatic nodules, both chemically and in its resistance to enzyme action The lesion is usually said to be due to exposure, but we had one old lady of ninety who for various reasons had lived in hospital since the age of seven, and she had this change in a very striking degree—where she got the sunlight in a hospital in Leeds I do not know!

*Lansing* Well, apropos of your first point, there certainly is no issue on which to cross swords I don't believe that there is a rich bed of elastic tissue in normal skin, and indeed the illustration I showed of the abdominal surface showed a remarkable lack of elastic elements, at least in the pars papillaris, there was rather a coarse net deep in the reticular portion of the skin, but I think we would all agree that there is probably less than 1 per cent extractable elastin in skin on a dry weight basis

We differ very sharply as to what this material is that we see in senile elastosis This may be a matter of semantics or one of simple definition, but the fact is that this peculiar bed of material appears in the pars papillaris of human skin usually but not necessarily in exposed portions of the body This material stains like elastic tissue We have very few criteria for defining elastic tissue the best ones we have depend on tinctorial reactions Affinity for orcein is rather specific, and this

elastic tissue, with one rather recent exception (the heat denatured collagen), responds to it, but within an hour after exposure to elastase at a $pH$ of 9 0 the material that normally stains like elastic tissue in senile elastosis disappears entirely, it does not take up any of the elastic tissue dyes *And lastly, that material when exposed to the aniline blue or* any of the Mallory reagents for collagen is entirely negative

*Tunbridge* I take you up on two points there Collagen acted on chemically or enzymatically, for example with pepsin, will then take up stains which are said to be characteristic for elastin, so I think that

*Tunbridge* Well, we have been very careful, and Banga agrees with us on this entirely, that once you get rid of the polysaccharide, trypsin then has a most dramatic action

*Lansing* I would like to reaffirm that this is mate-
logically looks like
elastic tissue and
criteria we have f
presumptive evide

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c
o

*Comfort* And that is the material which you describe as elastin and Prof Tunbridge as modified collagen?

*Lansing* Yes.

*Tunbridge* I think under the electron

When I examined the slide in the light microscope I saw the same tremendous masses of "elastoid material" as Dr Lansing has already shown Using the low power of the electron microscope (Fig 1) we again see the same masses But with higher power (Fig 2) it becomes obvious that this material must be degenerated collagen and not degenerated elastic fibres Don't you think so?

*Lansing* But in between the bundles of collagen, do you think the tissue is actually empty there or do you think perhaps you have a rather fragmented section?

*Tunbridge* Of course, when one employs the electron microscope, obviously some of the spaces are artifacts due to the technique Some may be intracellular substances and matrix of which we know very little and cannot get rid of But I think that you can compare section with section I do not know if Prof Albertini will agree with me on that

*Albertini* Yes, I do

*Lansing* The very ...
by what I call sp...
and not ground ...
think that we are ...
the only way one ...
side—a thick sec... phase microscope and an adjacent serial section cut much thinner and introduced into the electron microscope to enable one to check area against area That's what we've been doing and we don't get empty spaces

*Cowdry* Dr Lansing, could you say a word about purifying the enzyme?

*Lansing* Using organic solvents under standardized conditions of precipitation, we have a material that seems to be free of trypsin, and is well along towards purification, but it still has a long way to go I think in another few months we may be able to talk more fully about it

*Verzár* I am glad that the discussion has now reached basic problems of the ageing of cells and even of proteins ...
work of Dr ...

... us to the basically important question of the changes of tissue adaptation which m...
in the ...



FIG. 1 (Albertini) Elastoid degenerated masses in the dermis (El micr 2470)



FIG. 2 (Albertini) Transformation from collagen into elastoid masses (El micr 1,000)

[To face page 108

tron microscope the struc
with its perfect fibres—in

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cal

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*Albertini* Yes, I do

side—a thick section in the phase microscope and an adjacent serial section cut much thinner and introduced into the electron microscope to enable one to check area against area That's what we've been doing and we don't get empty spaces

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*Verzár* I am glad that the discussion has now reached basic problems of the ageing of cells and even of proteins I think that the admirable

even in nerve I think that this leads to the basically important question of the changes of tissue adaptation which might be caused by changes in the proteins

I would also like to say that a lot of time was spent in our laboratory with elastase estimations in young and old animals, and large differences in the pancreases of young and old animals were found but unfortunately they were not related to age.

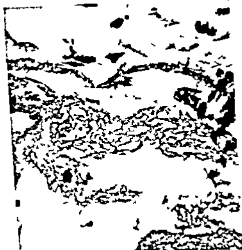


FIG 1 (Albertini) Elastoid degenerated masses in the dermis (El micr 2473)



FIG 2 (Albertini) Transformation from collagen into elastoid masses (El micr 15000)

[To face page 109

collagen fibres remaining So, under the electron microscope the structure is very different from ordinary collagen with its perfect fibres—in bundles and cross striations

*Albertini* May I show an electron microscopic pattern of a skin biopsy with senile keratosis and very typical so called elastosis which we also call "elastoid degeneration"

When I examined the slide in the light microscope I saw the same tremendous masses of "elastoid material" as Dr Lansing has already shown Using the low power of the electron microscope (Fig 1) we again see the same masses But with higher power (Fig 2) it becomes obvious that this material must be degenerated collagen and not

fragmented section?

*Tunbridge* Of course, when one employs the electron microscope, obviously some of the spaces are artifacts due to the technique Some may be intracellular substances and matrix of which we know very little and cannot get rid of But I think that you can compare section with section I do not know if Prof Albertini will agree with me on that

*Albertini* Yes, I do

*Lansing* The very low level of electron density in the area represented

side—a thick section in the phase microscope and an adjacent serial section cut much thinner and introduced into the electron microscope to enable one to check area against area That's what we've been doing and we don't get empty spaces

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of the changes of tissue adaptation which might be caused by changes in the proteins

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they were not related to age

# *CALCIUM METABOLISM IN OLD AGE AS RELATED TO AGEING OF THE SKELETON

O J Malm m d, R Nicolaysen, m d, and L Skjelkvale
*Inst for Ernæringsforsking Oslo*

Ageing of the skeleton implies dissolution of structural units (osteons and trabeculæ) with loss of calcium salts from the body and with a reduction of the breaking strength It is not known if the breaking strength is always proportionate to density of mineralisation

The systematics of bone diseases have been discussed in a number of text books and monographs, for example Albright and Reifenstein (1948)

Senile osteoporosis is said to be frequent in old age, but no good statistics have been produced The problem is how, if at all this disease is related to dietary intake of calcium and vitamin D Further, the course of calcium metabolism is not known during the development of the disease, but it must be negative over a period of years

Recently the problem has in part been ably discussed by Hegsted Moscoso and Collazos (1952) who consider that the calcium requirement of adult man is low and that the frequent occurrence of osteoporosis is not related to calcium intake

On the other hand numerous discussions of the Ca requirement of man have ended with a recommendation for a defined intake Lately the U S Food and Nutrition Board revised their recommendation of 1 g to a minimum of 0 8 g daily for adult men The implication must be that adult people consuming considerably less Ca than is recommended run the risk of getting into continuous negative Ca balance which in the course of time must lead to recognizable osteoporosis

Such differences of opinion can only be settled by adequate

*This is to be considered as a preliminary report presented by Prof. Nicolaysen.

The physiology of calcium metabolism was recently reviewed by Nicolaysen, Eeg Larsen and Malm (1953), and the biochemistry and physiology of vitamin D by Nicolaysen and Eeg Larsen (1953) Reference is made in these publications to a number of aspects of the field

In Oslo we have for several years studied the calcium metabolism of rats, and we have also conducted a long term study in man The studies are in part finished, in part they are continuing The main purpose here is to report briefly on these experiments, which will be published in full at a later date

## Experiments in Rats

The object has been to study the adaptation of the absorption of calcium in adult and old rats Some of the characteristic results are reported below

*Experimental* Adult rats of known age taken from our stock colony and given a vitamin D free diet (842 g whole wheat, 100 g casein [Ca 'free' and fat free], 30 g dried brewers yeast, 10 g NaCl, 50 g $B_{12}$ "mixture", 8 g $KH_2PO_4$, 50 g $CaCO_3$) with a Ca content of 0 25 per cent At intervals the $CaCO_3$ is removed and the diet then *contains* 0 03–0 04 per cent Ca With the aid of 0 5 per cent Na oxalate added to this Ca poor diet the animals are at given intervals deprived of body calcium

The Ca absorption or the Ca balance is studied continuously In Figs 1 and 2 two experiments are reported, one on a D free diet to which vitamin D was added when the animals had suffered a measured loss of Ca, the second in very old rats who received 0 5 i u vitamin D daily throughout

### Results

The contrast between the group of rats given a vitamin D free diet (*Fig* 1) in the periods following Ca deprivation (but before vitamin D was given), and the rats given vitamin D throughout (Fig 2) is striking Thus rats without vitamin D can lose 15–20 per cent of their Ca and the net absorption still remains very low When vitamin D is given to such rats

and representative experimental work The problem of Ca *balance and Ca requirement of elderly organisms has been* subjected to a number of studies in recent years According to McCay (1952) various species tend to get into negative Ca balance when about *two thirds through the span of life* Lin and McCay (1953) found that they had trouble in maintaining metabolic equilibrium in old dogs unless the diet was relatively rich in calcium

Owen (1939) and Owen *et al* (1940) in studies in old osteoporotic men, observed a marked tendency for Ca retention Bogonoff *et al* (1953) studied the Ca balance in seven elderly men (sixty six to eighty three years of age) of whom three had osteoporosis High, intermediate, and very low levels of Ca intake were used in periods of three to four weeks The tendency to retention on high levels was small but the loss on the low level (100 130 mg daily) was also small (100 200 mg daily) In contrast Ackermann and Toro (1953*a*) in a study of the Ca balance over fifty to seventy days in eight old men given only a high Ca diet, concluded that seven of the men needed 18 5 mg Ca/kg body weight to maintain equilibrium The eighth subject lost 0 5 1 0 g Ca daily in spite of a very high level of Ca in the diet

In these experiments, as well as in earlier animal experi*ments, a high fæcal Ca loss was observed, in other words* absorption seemed to be deficient, whereas the urinary Ca in these old men was in the lower range of normal

Schilling and Laszlo (1951) studied various bone diseases among them seven senile osteoporotic cases The urinary Ca was low, but they retained somewhat less of an intravenously injected amount of Ca salt than the normal controls (personal communication)

In view of the fact (see below) that vitamin D is a dominant factor in Ca absorption in both adult and in old age, it is of interest to note that Ackermann and Toro (1953*b*) observed a substantially increased Ca absorption in their subjects following vitamin D administration Balance was achieved, but no retention, *in the person who lost 0 5–1 0 g Ca daily*

It is worth while reporting that the technique used in the above experiments matured in consequence of a great number of experiments in which adaptation (significantly increased absorption) could not be observed In these earlier experiments rats given vitamin D throughout were used Short

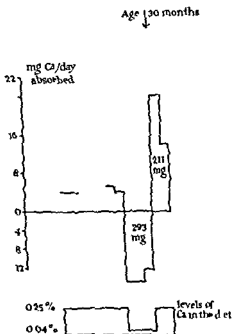



FIG 2 The Ca absorption in six old rats given 0 5 I U vitamin D daily (Ca intake on the 0 25 per cent Ca diet about 40 mg daily )

periods of ten to fourteen days on the 0 25 per cent Ca diet were used to test the absorption In between the rats were given the 0 04 per cent Ca diet for weeks and months Some suffered losses of 50–100 mg (2–4 per cent of the total), but this did not result in a clearcut increase of the absorption from the 0 25 per cent diet Other groups of rats very quickly reduced their fæcal output to very low values, so that they hardly lost any body Ca at all

the absorption quickly reaches very high values This result has been confirmed in a number of other experiments The results achieved in the very old (thirty one months) rats have so far been confirmed in fifteen to twenty months old rats

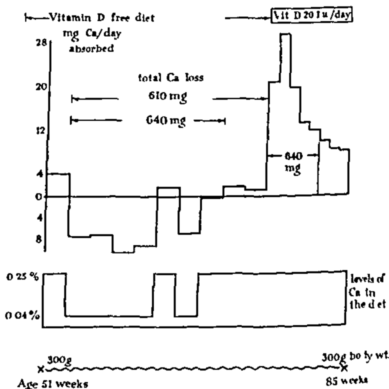
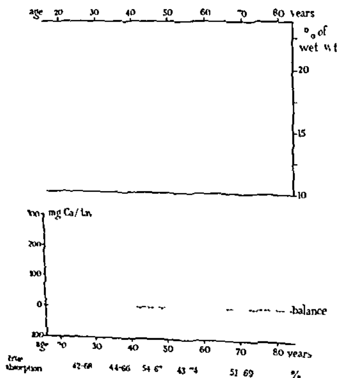



FIG 1 The absorption of Ca in six female rats (Ca intake on the diet containing 0 25 per cent Ca about 40 mg daily )

The urinary Ca was measured throughout in the very old rats The daily output was about 0 5 mg in the period of deprivation In the following two weeks it increased to about 2 mg daily Thus nearly all Ca absorbed was retained The loss of bone minerals suffered in the period of deprivation could quickly be made good

urinary and fecal Ca occurred when the intake was reduced. In such instances the standard deviation is an exaggeration of the error of the single observations.

The method of calculation used for estimated requirement rested on a number of assumptions and approximations




true absorption 42-68 44-66 54-67 43-74 51-69 %

True absorption calculated according to formula (Nicolaysen et al 1953 Physiol Rev 33 424)

$$\left(1 - \frac{\text{Ca (feces)}}{\text{Ca intake} + \text{Ca secreted*}}\right) 100$$

*500 mg used here

Fig. 3. Top: Ash content of whole corp. vertebrae in men killed by accidents.

Below: Calcium balances in 58 men. Continued observations over 100–500 days daily intake about 900 mg Ca

As a result, we introduced oxalate as a Ca "trapper"

It needs emphasis, that the lack of adaptation of the absorption in rats is in reality nothing but a confirmation of the principle which can be extracted from all earlier observations in vitamin D deficiency in man, not least from results in late rickets and in osteomalacia (see Nicolaysen and Eeg-Larsen, 1953, for the metabolic pattern)

## Experiments in Men

These were long term balance experiments conducted in our main prison Only several consecutive two week periods were used for calculations, and all observations were based on analyses of food as served and eaten The procedure was that any person taken for experiment was first observed for a period ranging from some months to a year on the level of Ca intake of about 900 mg daily When the Ca "characteristic" of a given person had thus been established at this level, the Ca intake was reduced, The vitamin D intake was at least 200 i u daily

## Results

In all thirty eight men were studied However, some were released before an adaptation experiment could be conducted over a sufficient period of time Therefore only twenty five out of the thirty eight were subjected to such a procedure

In Fig 3 the balances of all thirty eight men have been given, and in Table I are condensed and summarized the results of the adaptation study in twenty five men In Fig 3 the figures have been arranged according to age, whereas in Table I they are according to increased calculated requirement

Most of the men were overweight according to the Metropolitan Insurance Company's standard table which was used for correction

The figures for urine and fæcal Ca are given with their standard errors In some the variations were irregular, but in other persons long waves in the fæcal Ca output occurred at one level of intake A definite trend of the curves for

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 4041 Pronounced osteoporosis | 57 | 71 7+ | 70<br>84 | 1015<br>704 | 30<br>−.6 | 2 5<br>−2 2 | 0 - | 43<br>42 | 94± 4 1<br>76± 2 1 | 775±45 0<br>748±12 1 |
| 150 Slight osteoporosis columns pelvis | 58 | 69 4+ | 252<br>140<br>140 | 801<br>520<br>510 | 150<br>10<br>62 | 31 0<br>1 4<br>9 0 | 0 2 | 67<br>72<br>75 | 280± 5 2<br>225± 4 1<br>200± 8 1 | 455±12 0<br>285±19 0<br>218±10 4 |
| 502 n | 40 | 70 3+ | 210<br>140<br>120<br>140<br>98 | 942<br>421<br>430<br>450<br>437 | 19<br>−100<br>−45<br>8<br>−14 | 21 0<br>−15 0<br>−5 0<br>1 1<br>−1 3 | 0 4<br>0 5 | 57<br>65<br>69<br>74<br>80 | 238± 9 0<br>205± 4 7<br>203±14 7<br>202± 7 2<br>201±14 2 | 605±21 2<br>322±20 7<br>200±10 0<br>210±14 7<br>187±15 7 |
| 323 n | 20 | 70 0− (1 kg) | | | | | | | | |
| 321 n | 32 | 73 0− (3 kg) | 182<br>84 | 935<br>309 | 73<br>−57 | 12 0<br>−5 0 | 6 6 | 57<br>71 | 272±13 0<br>105± 3 6 | 610±17 5<br>260± 5 7 |
| 221 No X ray | 42 | 77 1+ | 98<br>120 | 970<br>401 | 87<br>−72 | 9 0<br>−4 0 | 6 6 | 51<br>68 | 170± 2 7<br>184± 8 0 | 710±21 5<br>309±21 9 |
| 604 n | 23 | 69 0+ | 252<br>84<br>98 | 965<br>450<br>469 | 137<br>−58<br>0 | 35 0<br>−3 0<br>1 0 | 7 1 | 54<br>62<br>71 | 140± 5 2<br>140±11 8<br>180± 7 7 | 682±25 0<br>302±23 2<br>280±14 7 |
| 203 n | 55 | 74 4+ | 98<br>70 | 981<br>454 | 20<br>−40 | 2 0<br>−3 0 | 7 1 | 46<br>63 | 105± 5 0<br>150± 5 1 | 790± 7 1<br>350±31 4 |
| 622 n | 37 | 63 1+ | 112<br>98<br>104 | 971<br>450<br>453 | −26<br>−111<br>−10 | −3 0<br>−16 0<br>−2 0 | 7 3 | 46<br>55<br>67 | 178± 8 9<br>137± 7 5<br>140± 5 8 | 770±29 3<br>424±28 1<br>314± 9 5 |
| 850 n | 42 | 65 8− (7 kg) | 224<br>112<br>84<br>70 | 929<br>426<br>430<br>801 | 0<br>−82<br>−37<br>81 | 0<br>−9 0<br>−3 0<br>6 0 | 7 3 | 54<br>73<br>78<br>80 | 272±10 3<br>254± 9 3<br>200± 7 9<br>258±15 6 | 657±42 3<br>254±20 5<br>207±30 5<br>552±40 2 |

n = X ray normal

+ = overweight
± = correct weight
− = underweight

*Calculated daily requirement per kg correct body weight

Table I

LONG TERM CALCIUM BALANCE STUDIES IN MEN

| No | Age years | Corrected body wt kg | Time days | Intake mg day | Balance mg/day | Balance total g | Cr* mg/l p c b | Calc per cent absorbed | Urine mg day | Faeces mg day |
|---|---|---|---|---|---|---|---|---|---|---|
| 273 slight | 64 osteo | 70 4+ poros is | 182 | 931 | 29 | 5 5 | | | 213 ± 10 2 | 709 ± 76 0 |
| | | | 84 | 349 | −20 | −1 7 | 4 8 | 72 | 137 ± 3 4 | 232 ± 32 3 |
| 790 n | 50 | 74 4+ | 140 | 910 | −8 | −1 1 | | 33 | 258 ± 3 8 | 662 ± 18 0 |
| | | | 112 | 1418 | 20 | 2 9 | | 40 | 251 ± 1 1 | 1139 ± 53 9 |
| | | | 140 | 452 | −61 | −8 5 | | 68 | 204 ± 4 0 | 300 ± 15 4 |
| | | | 140 | 428 | −4 | −0 7 | | 72 | 171 ± 8 1 | 261 ± 9 2 |
| | | | 126 | 368 | −7 | −0 9 | 5 1 | 77 | 169 ± 1 0 | 206 ± 15 8 |
| | | | 126 | 337 | −64 | −7 9 | | 72 | 171 ± 4 2 | 230 ± 21 5 |
| 205 n | 30 | 70 5+ | 84 | 419 | −1 | 0 | 5 2 | 72 | 158 ± 9 2 | 264 ± 14 6 |
| 164 n | 64 | 72 6+ | 168 | 968 | 165 | 28 0 | | | 215 ± 12 1 | 588 ± 15 9 |
| | | | 126 | 443 | 44 | 5 7 | 5 3 | 70 | 190 ± 6 8 | 200 ± 12 1 |
| 121 n | 31 | 67 0+ | 448 | 970 | 102 | 46 0 | | | 234 ± 5 8 | 634 ± 10 3 |
| | | | 168 | 430 | 49 | 7 0 | 5 7 | 71 | 170 ± 8 8 | 222 ± 16 3 |
| 92 n | 43 | 65 0+ | 532 | 953 | 49 | 26 0 | | | 410 ± 7 0 | 189 ± 12 3 |
| | | | 350 | 436 | 70 | 25 0 | 5 7 | 86 | 274 ± 8 2 | 132 ± 5 2 |
| 242 Osteoporosis? | 46 | 85 0+ | 336 | 950 | 180 | 64 0 | | | 367 ± 12 7 | 394 ± 20 0 |
| | | | 208 | 936 | 70 | 19 0 | | 53 | 217 ± 2 9 | 640 ± 12 4 |
| | | | 266 | 580 | −75 | −20 0 | | 57 | 192 ± 3 9 | 163 ± 11 6 |
| | | | 140 | 608 | 1 | 0 | 9 5 | 84 | 209 ± 5 0 | 398 ± 10 2 |
| | | | 266 | 416 | −46 | −16 0 | 7 5 | 68 | 170 ± 4 1 | 292 ± 12 9 |
| | | | 140 | 355 | −19 | −2 5 | 5 8 | 76 | 118 ± 4 1 | 200 ± 8 1 |
| 311 n | 28 | 78 5+ | 98 | 422 | −30 | −3 5 | 5 9 | 77 | 231 ± 13 7 | 207 ± 26 1 |
| 345 n | 30 | 71 0+ | 84 | 402 | −28 | −2 4 | 6 0 | 69 | 190 ± 0 9 | 240 ± 19 1 |
| 279 n | 54 | 78 5+ | 98 | 967 | 32 | 3 2 | | 50 | 190 ± 5 3 | 736 ± 13 6 |
| | | | 84 | 450 | −18 | −1 7 | 6 1 | [illegible] | 170 ± [illegible] | [illegible] |

One assumption obviously not completely valid was that adaptation had been fully developed and that it had reached its maximal power The three approximations used were the following

1 The true percentage absorption was calculated according to formulæ (see Fig 1 and Nicolaysen Eeg Larsen and Malm 1953)

2 It was assumed that this calculated percentage would be the same when the level of intake varied somewhat up or down

Two examples will illustrate the procedure

1 No 273 20 mg $\frac{100}{72}$ = 28 mg which added to the 849 mg results in the figure used

2 No 121 49 mg $\frac{100}{71}$ = 69 mg which subtracted from 450 mg results in the figure used

3 In a few persons a slight linear correction up or down of
the urinary Ca
correction neede
in 408c 25 mg

The balance was measured in fourteen men above fifty years of age and adaptation was studied in nine men above fifty years of age

## Discussion

### Absorption and its adaptation

It appears that the absorption is more efficient than usually thought The introduction of e g 300 and 900 mg respectively for the daily Ca content of the digestive juices will not materially alter this contention

It also appears that the power of absorption is increased in the course of time in practically all persons studied This contention rests on the assumption that the digestive juice Ca is not systematically altered in a period of negative balance

Table I—continued

LONG TERM CALCIUM BALANCE STUDIES IN MEN

| No | Age years | Corrected body wt kg | Time days | Intake mg/day | Balance mg/day | Balance total g | Ca r.* mg/kg c.b. | Calc per cent absorbed | Urine mg/day | Faeces mg/day |
|---|---|---|---|---|---|---|---|---|---|---|
| 288 n | 29 | 67.1+ | 272 | 913 | 55 | 15 | | 50 | 160± 3.8 | 608±11.3 |
| | | | 168 | 785 | 27 | 4 | | 55 | 185± 5.2 | 573±15.0 |
| | | | 98 | 508 | −53 | −5 | | 59 | 177± 8.9 | 444± 8.2 |
| | | | 84 | 584 | 23 | 2 | 8.2 | 63 | 158±10.4 | 403±11.3 |
| 408c n | 53 | 74.8+ | 182 | 923 | 101 | 20 | | 62 | 268±14.4 | 540±22.7 |
| | | | 140 | 479 | −80 | −11 | 8.2 | 68 | 239± 9.2 | 320±17.3 |
| | | | 126 | 504 | −77 | −10 | 8.8 | 61 | 180± 0.9 | 395±15.5 |
| 501 n | 31 | 68.0+ | 196 | 955 | 95 | 18 | | 56 | 217± 4.4 | 643±10.7 |
| | | | 140 | 421 | −112 | −14 | 8.9 | 62 | 190± 6.9 | 343±21.5 |
| | | | 84 | 451 | −79 | −7 | 8.4 | 67 | 212± 2.9 | 318±15.0 |
| 200 Slight osteoporosis columna pelvis | 50 | 62.1± | 210 | 909 | 121 | 25 | | 74 | 417±21.7 | 371±13.1 |
| | | | 224 | 595 | −46 | −10 | | 79 | 409± 8.9 | 232±11.8 |
| | | | 98 | 580 | 23 | 3 | 9.0 | 84 | 388±11.6 | 175±11.3 |
| 331 Osteoporosis? | 48 | 69.4+ | 294 | 890 | 0 | 0 | 12.8 | 59 | 320±10.3 | 570±11.4 |
| | | | 168 | 757 | −45 | −7 | 11.8 | 64 | 354± 3.8 | 448±11.3 |
| | | | 280 | 547 | −104 | −29 | 9.9 | 72 | 358±10.0 | 289± 7.8 |

n = X ray normal

\+ = overweight
± = correct weight
− = underweight

* Calculated daily requirement per kg corrected body weight

The approximations used cannot lead to serious errors affecting the main results

In the main most of the figures for Ca requirement support the view of Hegsted *et al* (1952) that Ca requirement for maintenance is low in men On the other hand the higher figures reached are at variance with such a view and some comments on these figures are needed The experiment in No 551 over twenty six months leads to a comparatively well established figure of 12 8 mg Is he an exception in the material presented? In view of the results in No 242, the figure reached in No 288 can be quite off the mark, and the figure reached for No 408c is not well founded due to the "abnormal' figure for fæcal Ca in the third period In the course of time this high fæcal output would have been followed by an inverse wave, with a much lower figure for Ca requirement as the result On the other hand the figure of 9 mg for No 200 would seem to be not too poorly founded Elementary statistics on the requirement figures for the first 24 persons results in a mean of 6 8 mg /kg with s d of 2 1 This would imply that No 551 is one in a thousand but it does not solve the problem

The men above fifty years of age seem not to behave differently as regards adaptation, and it appears from Fig 3 that a number of them retained Ca remarkably well The development of osteoporosis implies loss of calcium from the skeleton, either by a real increase of urinary Ca, or by a non reduction of urinary Ca combined with faulty absorption

The results in No 790 on the highest intake level are instructive as to how the absorption may seem to be low when only high levels of Ca in the diets are used, and thus may throw some additional light on the apparent faulty absorption in the old persons of Ackermann and Toro (1953) The results of [illegible] s of Ca in the diet [illegible] etween individuals [illegible] it impossible to judge if the urinary Ca in a given person has been increased Observations over many years

turning positive Anyhow, it seems reasonable in view of the undoubtedly established adaptation of absorption in rats, to assume that an increase of the power of absorption is responsible for most of the adaptation of the absorption

Persons above fifty years of age show on an average no less ability to adapt their absorption than younger persons The results of Bogonoff *et al* (1953) in old men on very low Ca diets strengthened this view

## Urinary Ca and its adaptation

The quantitative aspects have recently been discussed (Nicolaysen *et al*, 1953), and the linearity within individuals as well as the variability between individuals has been stressed

In a few persons no reduction of the urinary output follows halving of the intake, but in most persons some reduction follows, and in some a substantial reduction is responsible for quite a large part of the quick achievement of balance following the reduction of Ca intake

However, when two consecutive periods on a low level of intake are compared, the general impression is that absorption improves in nearly all, whereas the urinary Ca level mostly remains horizontal

## Ca requirement

The validity of the figures rests entirely upon the method of study The material here presented reveals the number of weaknesses inherent in such a study of arbitrarily chosen subjects of mostly unknown previous dietary habits and status of nutrition as regards Ca and vitamin D Judged from the number of persons able to retain substantial amounts of Ca it may be said that they did not represent a population well nourished as regards nutrients essential to maximal skeletal calcification On the other hand such a contention would lend support to the hypothesis that the limit of adaptation may have been reached in some

The approximations used cannot lead to serious errors affecting the main results

In the main most of the figures for Ca requirement support the view of Hegsted *et al* (1952) that Ca requirement for maintenance is low in men On the other hand the higher figures reached are at variance with such a view and some comments on these figures are needed The experiment in No 551 over twenty six months leads to a comparatively well established figure of 12 8 mg Is he an exception in the material presented? In view of the results in No 242, the figure reached in No 288 can be quite off the mark, and the figure reached for No 408c is not well founded due to the "abnormal figure for fæcal Ca in the third period In the course of time this high fæcal output would have been followed by an inverse wave, with a much lower figure for Ca requirement as the result On the other hand the figure of 9 *mg for No* 200 would seem to be not too poorly founded Elementary statistics on the requirement figures for the first 24 persons results in a mean of 6 3 mg /kg with s d of 2 1 This would imply that No 551 is one in a thousand but it does not solve the problem

The men above fifty years of age seem not to be [illegible] opment of osteoporosis implies loss of calcium from the skeleton, either by a real increase of urinary Ca, or by a non reduction of urinary Ca combined with faulty absorption

The results in No 790 on the highest intake level are instructive as to how the absorption may seem to be low when only high levels of Ca in the diets are used, and this may throw some additional light on the apparent faulty absorption in the old persons of Ackermann and Toro (1953) The results of Bogonoff *et al* (1953) on very low levels of Ca in the diet are in line with the results here presented

As regards urinary Ca the variability between individuals makes it impossible to judge if the urinary Ca in a given person has been increased Observations over many years

would be required On the other hand the considerable material of Ohlson *et al* (1952) in women can be included in the discussion, and this has recently been done (Nicolaysen *et al*, 1953) Nothing indicates that urinary Ca is increased in elderly men and women However, such a contention is far from conclusive, because comparison between individuals suffers from great variability No 200 as well as No 531 were not only high urinary excretors, but they seemed to keep the high urinary Ca level with tenacity It may be that they represent the type that in the course of time may develop senile osteoporosis with hospitalisation as a consequence

The two different types of observations presented in Fig 3 may indicate that the men above fifty, who retained Ca so well, did so because they had been somewhat demineralized in the years prior to the actual balance study

Nothing in the material here presented indicates that the Ca metabolism of men above fifty differs from that in younger men On the other hand the limitations of the material are obvious, so that it does not allow of any final conclusion as regards the origin of senile osteoporosis as related to dietary intake of calcium and vitamin D

It does not seem profitable to continue to work with an arbitrarily chosen material, however, adequate balance studies in a selected material of senile osteoporotics combined with the load method of Schilling and Lazslo (1951) might in the course of some years contribute definitely to the solution

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## DISCUSSION

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repair but not a complete one, but it may be that the periods of observation are not long enough

*Brull* I have done many calcium balances, and I have now some doubt about the value of them at all If you take a case of osteoporosis, and do the calcium balance you very often find normal requirements If you investigate their background, you find that some people who become osteoporotic have had a normal calcium intake, and others, who have no disease, had a low calcium intake Then there is the striking fact that when you take a patient who has just a normal balance but who has osteoporosis, and you give him very small doses of follicular hormone, [illegible]

The amount of phosphates in urine is above that in the fæces when the intake of vitamin D is sufficient, and the fæces content goes above that of the urine when the intake of vitamin D is below normal requirements. As soon as you give the necessary amount of vitamin D (about 4 μg /day) then the proportion of phosphates in the urine is above the phosphate in the fæces. That is a very sensitive method in man. In vitamin D deficiency, when practically no phosphates are absorbed, vitamin D can produce a tremendous increase in phosphate absorption with no effect on calcium.

*Nicolaysen* I am a little sceptical of that method. I have done a great many studies on the ratio of calcium to phosphate in the fæces and it only tells me that if there is no phytic acid in the fæces the phosphate varies with the calcium.

*Aub* In the fæces, yes, but not in the urine. Phosphate in the urine is a very complicated problem—calcium excretion appears simpler, or at least is better understood. There may be a very low phosphate in the blood stream but a very large excretion in the urine and the reverse situation may also occur. The differences appear to be dependent largely on the amount of reabsorption in the renal tubules.

*Brull* May I remind you that I demonstrated a threshold for phosphate about twenty five years ago, and the influence of hormones and other factors upon it?

*Nicolaysen* If you were to accumulate the phosphates in the fæces in a soluble form you would soon get diarrhœa.

*Aub I agree that phosphate excretion appears to depend on the* amount of calcium in the fæces. It appears in the fæces largely as dibasic calcium phosphate but it doesn't always appear in the urine in that way, the excretion there appears more complicated.

*McCay* It is interesting that you have found vitamin D of importance. We have tried for many years, off and on, and we have never been able to show a vitamin D requirement in old rats. You have different diets and conditions and it shows that results vary enormously under different experimental conditions.

*Nicolaysen* We have conducted a life time study in rats which is instructive on this point. The diet was free of vitamin D but when vitamin D was added optimal growth was seen (in the D free rats the final bodyweight was 10 per cent lower). The calcium balance was studied continuously over eighteen months. The difference in the *absorption of calcium between the +D group and the D free group* disappeared after twelve weeks of experiment. The total calcium retention per 100 g body weight was about 10 per cent lower in the D free rats. Vitamin D given to such D free rats resulted in a considerably increased absorption. This was true for rats at the age of thirty five weeks as well as at seventy five weeks. The amount of Ca absorbed was many times higher than that seen in rats of the same age which had been given vitamin D throughout.

*McCay* Absorption from the intestine is confirmed very well by radioactive calcium work. There seems to be no difference in absorption of radioactive calcium from the tracts of young and old dogs or young and

some other source of calcium like bone meal. So there must be other factors in milk that modify this utilization of calcium in old age, don't you think?

*Nicolaysen.* In three years of preliminary experiments we studied the

on successively in a staircase like way. Thus adaptation had been clearly developed. Vitamin D was given to all these rats throughout.

*Aub.* If you are looking for osteoporosis, why do you use men instead of women?

# 17-KETOSTEROID EXCRETION IN AGEING SUBJECTS*

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MANY studies of the changes in adrenal function with increasing age or with disease have been based on the indices of total urinary corticoid excretion and total urinary 17-ketosteroid excretion. They have demonstrated a decreased excretion of both classes of steroid with increasing age. However, these decreases are not parallel. To cite only one study, that of Pincus, Romanoff and Carlo (1954), carried out at the Worcester Foundation on men and women aged twenty to ninety, it was found that the 17-ketosteroids decreased more markedly with age than did the corticoids.

Two recent developments have made it possible to derive more information about the function of the adrenal gland in aged individuals from a more detailed study of urinary 17-ketosteroids. First, metabolism studies with the steroid hormones have established the relationships between endogenous steroids and the individual 17-ketosteroids of the urine, and secondly, methods have been developed which make possible the quantitative determination of six individual α-17-ketosteroids in a urine extract containing as little as 2.5 mg. of Zimmermann reacting material.

The metabolic relationships for which there is experimental evidence are presented in Figs. 1 and 2. Fig. 1 shows what we consider to be the principal pathways in the biosynthesis of the biologically active adrenal steroids. Cholesterol, a $C_{27}$ steroid with the $3\beta$-hydroxy-$\Delta^5$ configuration, and other

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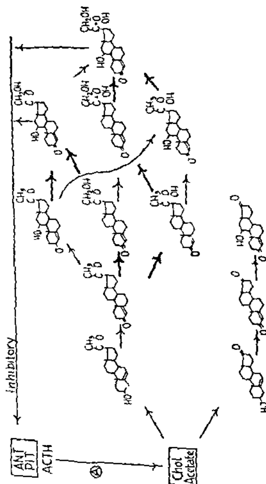



Fig. 1 Principle pathways in biosynthesis of biologically active adrenal steroids

precursors, such as acetate, first are converted to androst-5-en-3β-ol-17-one (dehydroepiandrosterone), a $C_{19}$ steroid, and pregn-5-en-3β-ol-20-one, a $C_{21}$ steroid, both still having the 3β-hydroxy-$\Delta^5$ structure. These are then oxidized to the corresponding $\Delta^4$-3-ketones, such as androst-4-ene-3,17-dione and pregn-4-ene-3,20-dione (progesterone). Oxygenation at carbons 11, 17, and 21 takes place after the oxidation of ring A, giving rise to androst-4-en-11β-ol-3,17-dione in the $C_{19}$ series, and in the $C_{21}$ series through six compounds containing, in various combinations, one or two more hydroxyl groups than progesterone, to the tri-hydroxy steroid pregn-4-ene-11β, 17α,21-triol-3,20-dione (cortisol). Among the compounds intermediate between progesterone and cortisol, those which can contribute to the urinary 17-ketosteroids are pregn-4-en-17α-ol-3,20-dione (17-hydroxyprogesterone), pregn-4-ene-11β, 17α-diol-3,20-dione, and pregn-4-ene-17α,21-diol-3,20-dione (11-desoxycortisol).

Fig. 2 shows the relationships between these steroids and the urinary 17-ketosteroids. Androsterone and ætiocholan-3α-ol-17-one excretion reflect the adrenal production of the $C_{19}O_2$ steroids, androst-4-ene-3,17-dione and dehydroepiandrosterone (and in the male, testosterone produced by the gonads), with a minor contribution from $C_{21}O_4$ steroids, such as 11-desoxycortisol. An indication of the production by the adrenal of $C_{19}O_3$ steroids, such as androst-4-en-11β-ol-3,17-dione, can be obtained from the urinary excretion of 11-oxygenated steroids having the 5α (or androstane) configuration, while excretion of 11-oxygenated 5β steroids (ætiocholane configuration) may be regarded as an index of *production of $C_{21}O_5$ steroids, the major one being cortisol.*

The quantitative method used at the Worcester Foundation for analysing urine extracts may be briefly described. Urines were collected as twenty-four hour specimens and hydrolyzed by boiling for eight minutes with 15 volumes per cent of hydrochloric acid. They were then extracted with ether and fractionated according to the method of Pincus (1945). After separation with the Girard reagent (Girard

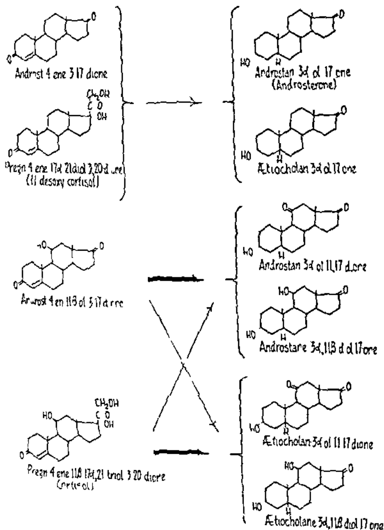



FIG 2 Relationships between adrenal steroids and urinary α 17 ketosteroids

precursors such as acetate, first are converted to androst 5 en 3β ol 17 one (dehydroepiandrosterone) a $C_{19}$ steroid and pregn 5 en 3β ol 20 one, a $C_{21}$ steroid, both still having the 3β hydroxy $\Delta^5$ structure These are then oxidized to the corresponding $\Delta^4$ 3 ketones, such as androst 4 ene 3,17 dione and pregn 4 ene 3,20 dione (progesterone) Oxygenation at carbons 11, 17, and 21 takes place after the oxidation of ring A, giving rise to androst 4 en 11β ol 3,17 dione in the $C_{19}$ series, and in the $C_{21}$ series through six compounds containing in various combinations, one or two more hydroxyl groups than progesterone, to the tri hydroxy steroid pregn 4 ene 11β 17α,21 triol 3,20 dione (cortisol) Among the compounds intermediate between progesterone and cortisol, those which can contribute to the urinary 17 ketosteroids are pregn 4 en 17α ol 3,20 dione (17 hydroxyprogesterone), pregn 4 ene 11β 17α diol 3,20 dione, and pregn 4 ene 17α,21 diol 3 20 dione (11 desoxycortisol)

Fig 2 shows the relationships between these steroids and the urinary 17 ketosteroids Androsterone and ætiocholan 3α ol 17 one excretion reflect the adrenal production of the $C_{19}O_2$ steroids, androst 4 ene 3,17 dione and dehydroepiandrosterone (and in the male, testosterone produced by the gonads), with a minor contribution from $C_{21}O_4$ steroids, such as 11 desoxycortisol An indication of the production by the adrenal of $C_{19}O_3$ steroids such as androst 4 en 11β ol 3,17 dione, can be obtained from the urinary excretion of 11 oxygenated steroids having the 5α (or androstane) configuration, while excretion of 11 oxygenated 5β steroids (ætiocholane configuration) may be regarded as an index of production of $C_{21}O_5$ steroids the major one being cortisol

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phenyl Cellosolve system to resolve androst 2(or 3) en 17 one (which arises from androsterone during the acid hydrolysis of the urine) 3β chloro androst 5 en 17 one and the acetate of ætiocholan 3a-ol 17 one (II) which is formed to a small extent during the Girard reaction The androsterone and ætiocholan 3a ol 17 one zones are eluted and oxidized with perbenzoic acid The oxidation products are rechromatographed and the 9 11 epoxides determined These arose from the 11β hydroxy steroids which were dehydrated during hydrolysis Chromatography for 72 hours in heptane propylene glycol of another aliquot of the extract or of material eluted from the paper strip in the area between the ætiocholan 3a ol 17 one zone and the starting line resolves the 11 keto compounds The composition of the fraction called residue is not yet fully determined Pr [illegible]

3a,17a dic [illegible]

20-one T [illegible]

including the zone for the ætiocholan 3a ol 11 17 dione, and therefore the determination of that compound may be somewhat high A few studies indicate that between 8 and 20 per cent of the Zimmermann value for the ætiocholan 3a ol 11 17 dione zone may be due to the presence of the 20 keto compounds

## Results

The daily excretion of a 17 ketosteroids of normal men and women is shown in Table I Though a change in excretion is seen in old age there is no significant difference between young men (nineteen to forty) and young women (twenty one to thirty five) or between old men (seventy to eighty eight) and old women (seventy four to eighty five) in total excretion or in the excretion of any single component In the younger age group the total a 17-ketosteroid excretion is 8 3–10 6 mg per day Of this 3 0–3 7 mg are present as androsterone, 2 6–3 9 mg as ætiocholan 3a ol 17 one 0 6-0 7 mg as

and Sandulesco, 1936) the digitonin non precipitable (or a) fraction of the ketonic extracts was obtained by the method of Butt, Henly, and Morris (1948) This fraction was dissolved in benzene and analysed by a quantitative paper chromatographic technique which has been described in

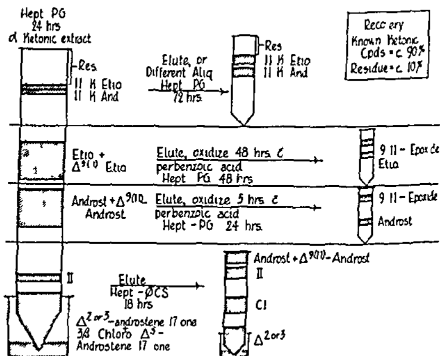



FIG 3 Scheme for quantitative paper chromatography of a ketonic urine extracts

detail (Rubin Dorfman and Pincus 1954) A brief summary of the analytical procedure is shown in Fig 3 An aliquot of the extract is chromatographed in the heptane propylene glycol system giving discrete zones of androsterone and ætiocholan 3a ol 17 one The materials whose mobilities on paper are more rapid than that of androsterone (both effluent material and material eluted from the paper strip distal to the androsterone zone) are rechromatographed in the heptane

phenyl Cellosolve system to resolve androst 2(or 3) en 17 one (which arises from androsterone during the acid hydrolysis of the urine) 3β chloro androst 5 en 17 one, and the acetate of ætiocholan 3α ol 17 one (II) which is formed to a small extent during the Girard reaction The androsterone and ætiocholan 3α ol 17 one zones are eluted and oxidized with perbenzoic acid The oxidation products are rechromatographed and the 9 11 epoxides determined These arose from the 11β hydroxy steroids which were dehydrated during hydrolysis Chromatography for 72 hours in heptane propylene glycol of another aliquot of the extract or of material eluted from the paper strip in the area between the ætiocholan 3α ol 17 one zone and the starting line resolves the 11 keto compounds The composition of the fraction called residue ' is not yet fully determined Preliminary studies indicate the presence of pregnane-3α 17α diol 20 one and possibly of allopregnane 3α,17α diol 20 one These two compounds may be dispersed over an area including the zone for the ætiocholan 3α ol 11,17 dione, and therefore the determination of that compound may be somewhat high A few studies indicate that between 8 and 20 per cent of the Zimmermann value for the ætiocholan 3α ol 11 17-dione zone may be due to the presence of the 20 keto compounds

## Results

The daily excretion of α 17 ketosteroids of normal men and women is shown in Table I Though a change in excretion is seen in old age there is no significant difference between young men (nineteen to forty) and young women (twenty one to thirty five) or between old men (seventy to eighty eight) and old women (seventy four to eighty five) in total excretion or in the excretion of any single component In the younger age group the total α 17 ketosteroid excretion is 8 3–10 6 mg per day Of this 3 0–3 7 mg are present as androsterone, 2 6-3 9 mg as ætiocholan 3α ol 17 one, 0 6-0 7 mg as

**Table I**
URINARY α 17 KETOSTEROID EXCRETION OF NORMAL MEN AND WOMEN
(mg /24 hrs ± S E )

| Sex | Age Range | Number of Subjects | Total α 17 KS | Androsterone | Etiocholan-3α-ol 17-one | Androstane 3α 11β diol 17-one | Androstan 3α-ol 11 17 dione | Etiocholane 3α 11β diol 17 one | Etiocholan-3α-ol 11 17 dione |
|---|---|---|---|---|---|---|---|---|---|
| M | 19–30 | 12 | 10.4 ± 0.9 | 3.7 ± 0.3 | 3.9 ± 0.6 | 0.69 ± 0.08 | 0.20 ± 0.03 | 0.25 ± 0.07 | 0.41 ± 0.04 |
| M | 31–40 | 8 | 10.6 ± 2.0 | 3.3 ± 0.6 | 3.7 ± 0.7 | 0.72 ± 0.11 | 0.32 ± 0.07 | 0.26 ± 0.07 | 0.46 ± 0.08 |
| M | 51–56 | 3 | 10.9 ± 4.7 | 4.1 ± 1.8 | 3.0 ± 1.2 | 0.43 ± 0.22 | 0.30 ± 0.13 | 0.21 ± 0.10 | 0.66 ± 0.20 |
| M | 70–84 | 5 | 3.4 ± 0.8 | 0.8 ± 0.2 | 0.9 ± 0.3 | 0.30 ± 0.07 | 0.10 ± 0.02 | 0.21 ± 0.04 | 0.21 ± 0.03 |
| F | 21–29 | 12 | 9.7 ± 1.0 | 3.1 ± 0.5 | 2.8 ± 0.4 | 0.56 ± 0.08 | 0.21 ± 0.03 | 0.24 ± 0.04 | 0.50 ± 0.08 |
| F | 32–33 | 4 | 8.3 ± 0.6 | 3.0 ± 0.5 | 2.6 ± 0.1 | 0.59 ± 0.20 | 0.18 ± 0.03 | 0.34 ± 0.10 | 0.52 ± 0.15 |
| F | 50–57 | 3 | 4.2 ± 1.1 | 1.2 ± 0.4 | 1.3 ± 0.6 | 0.28 ± 0.04 | 0.17 ± 0.04 | 0.10 ± 0.03 | 0.43 ± 0.02 |
| F | 74–83 | 8 | 3.2 ± 0.3 | 0.6 ± 0.1 | 0.9 ± 0.2 | 0.36 ± 0.04 | 0.10 ± 0.01 | 0.20 ± 0.05 | 0.33 ± 0.04 |

androstane $3\alpha$ $11\beta$ diol 17 one, 0 2–0 3 mg as androstan $3\alpha$ ol 11 17 dione, 0 24–0 34 mg as ætiocholane $3\alpha$ $11\beta$ diol 17 one and 0 41–0 52 mg as ætiocholan $3\alpha$ ol 11,17 dione These figures are in agreement with Dobriner (1953) except for ætiocholane $3\alpha$ $11\beta$ diol 17 one excretion

In older people the total excretion has fallen to 3 2–3 4 mg Androsterone and ætiocholan $3\alpha$-ol 17 one have decreased to 0 6–0 8 and 0 9 respectively The decreases in 11 oxygenated steroids were less severe Androstane $3\alpha$ $11\beta$ diol 17 one excretion was 0 30 0 37 mg androstan $3\alpha$ ol 11 17 dione excretion was 0 1 mg ætiocholane $3\alpha$ $11\beta$ diol 17 one was 0 2 mg and ætiocholan $3\alpha$ ol 11,17 dione was 0 21–0 23 mg per day

Only three men and three women in an intermediate age group (fifty to fifty seven) have been studied Though it would appear that the decrease in 17 ketosteroid excretion in the men appears only with more advanced age the impression is erroneous The high standard error for the male group indicates the wide variation and in fact one of the male subjects had a 17 ketosteroid excretion which was double that of the mean for the younger men The other two men showed the same degree of decline which may be seen for the women in the middle aged group

## Discussion

Since men and women of the same age group differ only slightly from each other in the excretion of any of the urinary α 17 ketosteroid components studied it appears that the contribution of testosterone to the urinary 17 ketostero[illegible]

[illegible] in androgenicity than the androgenic steroids of adrenal origin to determine the structural and functional differences between male and female

As shown in Fig 1 it is likely that the principal pathway of adrenocortical steroid biosynthesis is from the $3\beta$ OH $\Delta^5$ structure to the $\Delta^4$ 3 ketones either $C_{19}$ or $C_{21}$ and that 11,

17, and 21 oxygenation take place after the conversion to the $\Delta^4$ 3 ketone Analyses of urinary 17 ketosteroid excretion in subjects with adrenal hyperactivity have led us to the conclusion that those syndromes are due in part to imbalances in the concentrations of the various biosynthetic enzymes (Rubin *et al*, 1954 Dorfman 1954) It is also possible to discuss the variations from the young normal pattern which are seen in old age in terms of the same enzyme systems

In old age, androsterone and aetiocholan 3α ol 17 one excretion are decreased to about 20 30 per cent of the young adult level while 11 oxygenated 5α compounds are being excreted at about 50 per cent of the younger value and 11 oxygenated 5β steroids at about 60 per cent Therefore we must assume that the enzyme systems converting cholesterol acetate, or other precursors to dehydroepiandrosterone are the enzyme systems functioning at the lowest level in comparison with those of young adults This would account for the low excretion of 11 desoxy $C_{19}$ steroids The conversion from $\Delta^5$ 3β OH to $\Delta^4$ 3 ketone and the 11 oxygenating function are less impaired and such dehydroepiandrosterone as is formed is converted readily to androst 4 en 11β ol 3 17 dione $C_{21}$ steroids are also being formed and oxygenated at C 11 relatively efficiently as shown by excretion of two thirds of the normal young adult amount of 11 oxygenated 5β steroids

The connection between the indicated changes in production of individual adrenal hormones and the signs and symptoms of old age has not yet been established To this end, a study has been started at the Worcester Foundation, in collaboration with Dr Eric Bloch and with Dr Harry Freeman and the research staff of the Worcester State Hospital, in which twelve elderly men are to be given for many months, steroids calculated to restore the urinary α 17 ketosteroid pattern to that seen in young men Concurrently periodic tests are made of recent memory, as an index of mental function and of weight lifting ability, as an index of muscle performance The daily sublingual dose of steroids consists of 10 mg of

testosterone, 5 mg of androst 4 ene 3,11,17 trione (adrenosterone), 7 mg of cortisol acetate, and 2 mg of pregn 4-ene 11β,21 diol 3,20 dione (corticosterone) On this regime, the mean total 17 ketosteroid excretion increased from 3 5±0 4 mg per twenty four hours to 15 8±1 2 mg The urinary excretion pattern has so far been determined for only one subject It is shown in Table II Total 17 ketosteroid excretion was 21 8 mg, of which 9 mg were androsterone, 6 6 mg

Table II

URINARY α 17 KETOSTEROID EXCRETION OF A SEVENTY SEVEN YEAR OLD MAN TREATED WITH STEROIDS *

mg /24 hrs

| | |
|---|---|
| Pre treatment total 17 KS | 4 2 |
| Treatment total 17 KS | 21 8 |
| Androsterone | 9 0 |
| Ætiocholan 3α-ol 17-one | 2 6 |
| Androstane 3α 11β-diol 17-one | 0 4 |
| Androstan 3α-ol 11 17-dione | 0 22 |
| Ætiocholane 3α 11β-diol 17-one | 0 14 |
| Ætiocholan 3α-ol 11 17-dione | 0 58 |

*Daily Sublingual Dose = 30 mg Testosterone
= 5 mg Adrenosterone
= 7 mg Cortisol
= 2 mg Corticosterone

were ætiocholan 3α ol 17 one 0 4 mg was androstane 3α,11β diol 17 one 0 22 mg was androstan 3α ol 11,17 dione, 0 14 mg was ætiocholane 3α 11β diol 17 one, and 0 58 mg was ætiocholan 3α ol 11,17 dione The total 17 ketosteroids and the 11 desoxy $C_{19}$ steroids are higher than the mean for young men but within the range for young men The daily dose of testosterone is apparently too high The 11-oxygenated steroids approximate young normal values although the 11-oxygenated androstane derivatives are rather low

Apparently the daily dose of adrenosterone should be twice as great, or the steroid administered should be androst-4-en-11β-ol-3,17-dione, which was demonstrated by Romanoff, Hudson and Pincus (1953), to be the steroid produced by the adrenal gland. This fact was demonstrated after the establishment of the experimental regime for this study. It is as yet too early in the study to make a statement as to its probable outcome.

## Summary

Quantitative studies have been made of six α-17-ketosteroids in the urines of normal young men and women (aged nineteen to forty) and of normal old men and women (aged seventy to eighty-eight). There is no significant difference in the excretion of any of these compounds between men and women of the same age group. When the excretion of the steroids by old men and women is compared with that by young men and women, it is found that androsterone and ætiocholan-3α-ol-17-one excretion are decreased to 20–30 per cent of the values for the younger group, while excretion of 5α-11-oxygenated steroids is 50 per cent of that of the younger group and excretion of 5β-11-oxygenated steroids is 60 per cent of that of the younger group. If this difference be discussed in terms of those adrenal enzyme systems involved in converting cholesterol acetate or other precursors to the steroid hormones, it would appear that the enzyme systems functioning in old age at the lowest level in comparison with those of young adults are the ones which convert the precursors to dehydroepiandrosterone. The conversion from $\Delta^5$-3β-hydroxy to $\Delta^4$-3-ketone and the 11-oxygenating function are less impaired. $C_{21}$ steroids are also being formed and oxygenated at C-11 relatively efficiently. This is in agreement with those studies which have demonstrated a greater decrease with age in total 17-ketosteroid excretion than in corticosteroid excretion.

The significance of this finding with respect to the signs and symptoms of old age is a subject only for speculation.

at the present time, but a study is in progress in which recent memory and muscle strength tests are being taken at intervals by aged men on a regime of steroid administration designed to restore the urinary pattern to that of normal young men.

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DORFMAN [illegible]

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ROMANOFF, E B, HUDSON, P, and PINCUS, G (1953) *J. clin Endocrin* 13, 1546

RUBIN, B L, DORFMAN, R. I, and PINCUS, G. (1954) *Rec Progr Hormone Res.*, 9, 213


## DISCUSSION

*Tunbridge* Dr Freeman, would you like to elaborate Dr Rubin's last remark?

*Freeman* We gave 15 subjects a placebo for one month and then the potent [illegible]

[illegible] and we also had brain waves but these haven't been reported on yet The psychological tests showed absolutely no change from beginning to end, if anything there was a slight decrease in memory quotient This may be due to the natural ageing of the individual, and we will have to check this by runn[illegible] placebo group for th[illegible] the placebo an[illegible] not been [illegible]

in two to three months, ninety-five in four months, and staying at that level for the fifth month—an increase of about 84 per cent It may be

very well preserved men, and with

ght get more on the psychological

*Tunbridge* There is some work, I believe, to suggest that when old people are kept immobile there is a difference in excretory values from when they are mobile and active You've not done any studies on people in bed, have you?

*Rubin* Most of our subjects do live in an old people's home, but they are not bedridden

*Tunbridge* There is evidence from fracture cases, for what it's worth that the level when they are outside living in the country, for example farmers, is not so low When we bring them in after a fracture the level drops lower than in your cases

*Rubin* But with fracture cases you are running into the stress factor

*Olbrich* How do you exclude the increase in muscle strength by daily exercise?

*Freeman* The strength test is only run once a month, so there is no practice effect at all There was one session at the beginning of the placebo period, and one at the end, and the mean of each of the two tests was within a second of the other

*Olbrich* And what was the interval for the memory test?

*Freeman* That was done in a control period and three and five months after the initial level

*Comfort* Did they have subjective effects, either during the placebo period or during the medication?

*Freeman* I believe the placebo period was too short During the medication period some said they felt better and there were varying reports of increased or decreased urination The general report by the

25 per cent of cases show some improvement on placebos

*Mirscher* We spoke yesterday about the vitality factor Do you think

It was just

of muscular strength It could equally be regarded as a test of endurance Gord a O'Connor and Tizard found for example in defectives that if you give a test of this type, people may change in their endurance and consequently in their achievement They carry out the performance more and more effectively as their standard rises, or as they learn what they

achieved the last time and try to improve on it. It may just as likely be a psychological test as a physical one

*Freeman:* That is true As a matter of fact these people didn't know what their particular endurance was

the young men. But I was very much surprised at his result. The

that basis

*Parkes* But not between male and female?

*Rubin* No

*Krohn* Miss Rubin, about the precursor of all these hormones, you say it can be either cholesterol or acetate These two substances seem to be so different chemically that I do not see how you imagine any particular enzyme system coping with both of them

*Rubin* I was very careful to hedge about that. Dr. Hechter and his group are at present engaged on perfusing adrenals with various precursor substances, but the whole thing is very much in a state of flux I can only say that the major block seems to be in the production of dehydroepiandrosterone

peop

stres

exac

*Rubin* We did some work several years ago when we gave 25 mg. ACTH a day to various age groups, and we couldn't show any difference either in total excretion or in individual patterns We couldn't demonstrate any great response to the dose of ACTH at any age.

(1950) J. Geront., 5, 203

[1] Duncan, L. E., Solomon D. H., Rosenberg, E. F., Nichols, M. P. and Shock, N. W. (1952) J. Geront., 7, 351.

in two to three months ninety five in four months and staying at that level for the fifth month—an increase of about 64 per cent It may be that this group was a select group of very well preserved men and with a less well preserved group you might get more on the psychological side I don t know

*Tunbridge* There is some work, I believe, to suggest that when old people are kept immobile there is a difference in excretory values from when they are mobile and active You've not done any studies on people in bed, have you?

*Rubin* Most of our subjects do live in an old people s home but they are not bedridden

*Tunbridge* There is evidence from fracture cases for what it s worth that the level when they are outside living in the country for example farmers, is not so low When we bring them in after a fracture the level drops lower than in your cases

*Rubin* But with fracture cases you are running into the stress factor

*Olbrich* How do you exclude the increase in muscle strength by daily exercise?

*Freeman* The strength test is only run once a month so there is no practice effect at all There was one session at the beginning of the placebo period and one at the end and the mean of each of the two tests was within a second of the other

*Olbrich* And what was the interval for the memory test?

*Freeman* That was done in a control period and three and five months after the initial level

*Comfort* Did they have subjective effects either during the placebo period or during the medication?

*Freeman* I believe the placebo period was too short During the medication period some said they felt better and there were varying reports of increased or decreased urination The general report by the majority was of not feeling so tired I wouldn t trust this unless I ran a group on placebos where the investigator didn t know which was which There have been an increasing number of reports on the effect of placebos on pain and headache—I believe that in arthritis it has been shown that 25 per cent of cases show some improvement on placebos

*Miescher* We spoke yesterday about the vitality factor Do you think that by giving testosterone there is improvement of the physical vitality,

strength It could equally be regarded as a test of endurance Gordon

# TISSUE TRANSPLANTATION TECHNIQUES APPLIED TO THE PROBLEM OF THE AGEING OF THE ORGANS OF REPRODUCTION

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The interpretation that is attached to the word "ageing" depends very much on the particular tissue whose cells are ageing An individual neurone in the central nervous system, for example, may divide by mitosis until the end of the first year of life, it may increase in size later in life, and it is also capable of regenerating a new piece of fibre if its axon is cut An unknown proportion of such cells dies as time goes on, but seemingly none of them are ever replaced The components of other tissues, such as skin or the epithelium of the gut, are continually being replaced and the cells that one sees in an old animal are therefore, not old in the way that neurones are old, for they may just have been formed by the most recent of a series of cell divisions which stretch back into childhood and earlier Both tissues have aged but the individual cells that make up the tissues have not both aged in the same way

Other types of specialised tissue, e g the mammary gland or the uterus, undergo cycles of activity during the life of an individual The cells grow, mature, function, become atrophic or senile and then after an interval for recuperation may repeat the series of changes The functional capacity of the tissue alters as its experience of stimulation by hormones increases with each cycle of activity For the mammary gland this is demonstrated by the increasing amount of milk which is produced in successive lactations The previous experience of the uterus to hormone also conditions its subsequent sensitivity to further stimuli

when ACTH first reared its ugly head, there was considerable clinical opinion that older individuals responded to stresses of various kinds less effectively than the young, and presuming that ACTH represented a physiological stimulus to the adrenal cortex, it was thought that one ought to see differences in response to a physiological stimulus.

*Rubin*: I'd like to point out once more that in these old people the 11-oxygenated 17 [illegible] e not [illegible] duc [illegible] t lev [illegible] l 17- [illegible] dec [illegible] I think the physiological tests of metabolic response of which Dr. Shock speaks would be due to the 11-oxygenated $C_{21}$ steroids, so one would not expect a marked difference in young and old subjects.

closely related embryologically, the ovary may undergo considerable hyperplasia in utero which is followed later by degeneration and regression

More numerous papers emphasize the converse finding that the young postnatal ovary of various experimental animals is insensitive to hormones Absolute insensitivity of the ovaries of four week old rabbits has been reported by Hertz and Hisaw (1934) and Parkes (1943) has shown that female rabbits do not respond to a gonadotrophin preparation from horse pituitary glands until they weigh more than 1 3 kg Adams (1953) reports that ten week-old rabbits are insensitive to a similar type of pituitary extract whatever their weight Their capacity to respond increases rapidly during the next two weeks and super-ovulation can be achieved when a rabbit is three months old The ova which are released can be fertilised but fail to develop in the immature animals not, however, because the ova themselves are inadequate but because the necessary uterine conditions have not yet been achieved The ova will develop fully if they are transferred to the uterus of a mature host

The duration of this early phase of absolute insensitivity of the ovaries varies considerably from one species to another It appears to be very short in the rat (Price and Ortiz 1944) and the young heifer Marden (1951) has reported that a three-week-old calf will respond to injections of gonadotrophin at least as well as an eight to eleven month old calf On the other hand young female monkeys are insensitive for relatively much longer In general the interstitial tissue of the ovary responds at an earlier age than does the follicular apparatus, which seems to grow slowly but autonomously until the stage of antrum formation is reached

*(b) Changes at puberty*

In some species the pituitary gland contains gonadotrophin even before the animal is born The hormone gradually accumulates in the gland as the age of puberty is approached (Lawson Golden and Severinghaus 1939) but none is released

The ovary is a composite organ some of whose cells undergo cyclic changes similar to and in fact responsible for the changes in the mammary glands and uterus The other main group of cells the oocytes may, as will be seen later behave rather like some neurones Their lifespan may indeed rival that of neurones although the chance that any particular oocyte will survive as long is much less It is about the age changes in the ovary and the ways in which the methods of tissue transplantation can be used to study the changes that I wish to talk today

## The Life of the Ovary

### Temporal changes in the sensitivity of the ovary to its hormonal environment

(a) *During intra uterine life and before puberty*

A discussion of the process of ageing in the ovary cannot be restricted to the adult or old organ but must also consider the changes that take place during the prepubertal period The ovary can indeed be permanently affected by its hormonal environment very early in intra uterine life It is generally believed for instance that the ovary of the free martin female cattle twin develops abnormally because it is affected by the transplacental passage of hormones from its brother The gonads of a fœtal foal may at one stage be twice the size of the ovaries of the parent (Amoroso and Rowlands 1951) This enlargement due mainly to an increase in the amount of interstitial tissue is attributed to the effects of œstrogen rather than gonadotrophin because it occurs during the latter part of pregnancy when the serum gonadotrophin levels characteristic of the earlier stages have fallen and because serum gonadotrophin does not cross the placental membrane Baesich (1949 1950) has demonstrated regressive changes in the human ovary just after birth which he relates to the withdrawal at the time of delivery of a hormonal stimulus Like the adrenal cortex therefore to which it is so

clear nor is it certain for how long into old age the increased titres of gonadotrophin continue

It is certainly reasonable, at first sight to believe that the menopause develops in women because the ovary is no longer capable of responding to gonadotrophin but the evidence on this point is not very strong and even contradictory Kurzrok and Smith (1938) found that the response to treatment with a pregnant mare serum extract gradually decreased as the age of the patient increased The ovaries of two women aged sixty three and sixty six did not respond at all Westman (1934) on the other hand believed that he restored ovarian function in two women aged fifty one and forty eight who had stopped menstruating by giving them transfusions of blood from pregnant women which contained an anterior pituitary like gonadotrophin Waldeyer (1934) in a criticism of this work described seven menopausal women in whom ovarian function apparently restarted after an interval of four or five years without any treatment and referred to other reports that regular menstrual periods may recur seven or even nine years after the menopause Waldeyer also quoted the work of Tschertok and Penkow who treated seven menopausal women with extracts of pregnancy urine unsuccessfully

The disappearance of ova from the ovary does not necessarily mean that the post menopausal ovary is an entirely inert organ (see Wallart 1942) Woll Hertig Smith and Johnson (1948) also believe that the stromal elements may become hyperplastic as a result of stimulation by pituitary gonadotrophin and that the occurrence of carcinoma of the uterus can be correlated with such changes

As far as is known no such abrupt menopause occurs in other species but the information available is extremely scanty either because the animals die too soon in their natural habitat or are killed too soon for ... [illegible]

... menstruating in 1929 and which was still menstruating in 1947 (The animal is still alive and

At puberty the material which has been locked up in the pituitary is set free to act on ovaries which have also become progressively more responsive. The process which triggers the initial release is, however, altogether unknown If Kallas (1929) is correct, the immature gonads already hold the pituitary in check before puberty. He found that a 15-20 g rat in parabiosis with a castrated immature rat of the same weight rapidly came into œstrus. Kallas believed that the absence of the gonads in one parabiont allowed its pituitary to release gonadotrophin which stimulated growth in the gonads of the other We now know, however, that the non-specific stress of surgical operations may be sufficient to induce precocious puberty (Mandl and Zuckerman, 1951)

### (c) *During maturity*

The reproductive processes continue to mature after the first pregnancy and maximum efficiency, as determined by the number, size and development of the individual fœtuses, is not reached until several pregnancies have occurred This is true both of laboratory and agricultural animals Not only are the number of ovulations increased (either because more gonadotrophin is being secreted or because the ovaries are more sensitive) but the intra uterine conditions evidently become more satisfactory since the individual fœtuses are larger as well as more numerous

### (d) *The end of reproductive life*

The maximum reproductive capacity is maintained for several pregnancies before it begins to decline The most dramatic and rapid change is seen in women when the onset of the menopause, with which is associated a decreased production of sex hormones and the disappearance of oocytes from the ovaries, indicates the end of the child-bearing epoch Whether the increased amounts of gonadotrophin circulating in the blood and urine at this time represent extra production by the pituitary or decreased utilisation by the gonads is not

clear, nor is it certain for how long into old age the increased titres of gonadotrophin continue

It is certainly reasonable, at first sight, to believe that the menopause develops in women because the ovary is no longer capable of responding to gonadotrophin, but the evidence on this point is not very strong and even contradictory Kurzrok and Smith (1938) found that the response to treat ment with a pregnant mare serum extract gradually decreased as the age of the patient increased The ovaries of two women aged sixty three and sixty six did not respond at all Westman (1934) on the other hand believed that he restored ovarian function in two women aged fifty one and forty eight, who had stopped menstruating by giving them transfusions of blood from pregnant women which contained an anterior pituitary like gonadotrophin Waldeyer (1934) in a criticism of this work described seven menopausal women in whom ovarian function apparently restarted after an interval of four or five years without any treatment and referred to other reports that regular menstrual periods may recur seven or even nine years after the menopause Waldeyer also quoted the work of Tschertok and Penkow who treated seven meno pausal women with extracts of pregnancy urine unsuccessfully

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As far as is known no such abrupt menopause occurs in other species but the information available is extremely scanty either because the animals die too soon in their natural habitat or are killed too soon for econom[illegible]

Informat[illegible]

is es[illegible]

whic[illegible] menstruating in 1929 and which was still menstruating in 191[illegible] (The animal is still alive and

menstruating at rather irregular intervals in 1954 Whether it is ovulating or not is unknown ) Another baboon also menstruated over a period of at least fourteen years A monkey in the Birmingham colony accidentally died during pregnancy at an age of fourteen to fifteen and other spayed animals which are being used to compare the sensitivity to hormones of the uterus in youth and old age are now approximately twenty years old The only other information is provided by Hartman (1938) who mentions that his colony of rhesus monkeys contained two animals which reached the ages of eighteen and seventeen before they died Both were still menstruating but they had not ovulated for the preceding three and one and a half years respectively Since the menarche occurs at about the age of four these fragmentary observations suggest that reproductive life in monkeys may last for as long as twenty years a period which probably represents a greater proportion of the total lifespan than does the phase of reproductive life in women

## Strain differences in the rate of changes with age

The rate at which reproductive function comes to an end seems to vary considerably even within a single species Fekete (1953) has described the histological appearance of the ovaries of different aged mice from eight inbred strains Her observations are only qualitative but they show for instance that the number of follicles is noticeably diminishing in mice of the RIII and C57 strains when they have reached the age of eight months while similar changes have not taken place in the C3H strain at thirteen months of age Loeb (1948) also provides qualitative information about inter strain differences in the number of follicles and in the histological appearance of other components of the ovaries of mice

Some similar observations have been reported by Wolfe and Wright (1943) for two strains of rats which at equal ages differed not only in the relative proportions of the cell types in the pituitary but also in the numbers of normal and atretic follicles and of corpora lutea However Wolfe and

Wright only counted follicles over 0 3 mm. in diameter and therefore omitted all the smaller follicles which make up 90-95 per cent of the total population.

## The Age of Ova at the Time of Ovulation and the Problem of Oogenesis

It is becoming increasingly clear that the age of the mother at the time of ovulation may affect the future characteristics of the offspring in all sorts of ways Since most of the evidence bearing on this point has been brought together in a symposium organized by the New York Academy of Sciences (1954) there is no need to do more than mention a few examples. The increased risk of giving birth to a Mongol child when the mother is more than forty years old has long been recognised. The defect, whatever its cause, seems to be related to the age of the mother only and not to the number of pregnancies that she has had The chances of finding a wide range of embryological defects and the susceptibility of animals to neoplastic disease are also related to the age of the mothers (Strong, 1954, Law, 1954)

The relative significance of, on the one hand, the ageing ovum in the ovary, and the ageing uterine environment on the other, cannot yet be decided It remains important, therefore to determine whether the ovum that is fertilised should be regarded as a young or an old cell We are thus led immediately to consider the problem of oogenesis, which has formed one of the major points of controversy among *reproductive* physiologists for a very long time

Two entirely contrary views have taken it in turn to become the accepted dogma The older view is that the ovary is endowed during embryonic development with a fixed number of primordial germ cells from which the oocytes subsequently develop These cells are not derived from the germinal epithelium which covers the ovary, but migrate to the ovary before birth Multiplication by ordinary mitosis may occur during intra uterine life but has come to an end soon after birth, from then on the ovary merely uses up the material

that it already contains If this view is correct then the life span of an ovum may approach that of a neurone and one might expect that an ovum which was fertilised when a woman was eighteen years old would behave differently from one that was fertilised when she was forty years old Lansing's (1954) demonstration in rotifers that the lifespan and reproductive capacity of the offspring are greatly influenced by the age of the mother may well turn out to be relevant in this connection It is believed that a fixed number of future oocytes are incorporated into the ovaries of these animals during embryological development and that no further formation of oocytes takes place Hence the lifespan of the offspring depends directly on the length of life of the individual ovum before fertilization

The alternative view which has become more popular recently is that there is a continuous process of destruction and neoformation going on in the ovary throughout reproductive life It derives to some extent from the feeling that the signs of active atresia are so evident in histological sections of the ovary that neoformation must also occur to make up even partially for the great loss of cells Though a possible contribution of other elements has never been ruled out the cells in the germinal epithelium covering the ovary are usually thought to be the equivalent of the layer of spermatogonia in the testis and to be responsible for the replacement of the oocytes that are destroyed by atresia The rates of the two processes of oogenesis and spermatogenesis thus become much more similar It has been suggested indeed that an ovum lives no longer than a red blood cell and that its lifespan is to be measured in days rather than years

If this second view is correct an ovum that was released when a woman was forty would possibly have suffered no greater age changes than one released when she was eighteen and the effects of maternal age on the fœtus might depend more on changes in the uterine environment than in the ovary It would also mean that attempts to prolong reproductive life in women could be based not only on

controlling the destructive processes of atresia, but also on stimulating continued division in the cells of the germinal epithelium after they have ordinarily become inactive

There is no need to discuss fully the evidence for and against these two views since it has been summarised and criticised in detail by Zuckerman (1951) who supports the older view in his summing up Two things stand out The first is the extraordinary paucity of reliable counts of the total number of follicles in the ovary of any species at different ages, and the difficulty of forming a satisfactory picture of oogenesis by piecing together a series of discontinuous histological observations Second neoformation even if it occurs at all, is totally insufficient in women and partly insufficient in other animals to compensate for the atresia that takes place

## Age changes in the ovum

However long or short the life of an oocyte before its final maturation may ultimately turn out to be, the ovum certainly does not go on living for very long after ovulation and the present trend of [illegible]
during wh [illegible]
Probably [illegible]
even within this interval the ovum's capacity to develop into a normal embryo falls off progressively The work of Blandau and of Young (1953) has shown that, in both guinea pigs and rats, the proportion of embryos with defects of development increases as the time interval between ovulation and fertilisation is increased The ability of the ovum to become fertilised is not, however, altered in the same way. The proportion of fertilised ova in the tubes is not changed and it is only in the later stages of implantation and development that the influence of the ovum's age becomes apparent. This finding applies to cattle as well, according to Barrett (1948) who has studied the conception rates in dairy cows artificially inseminated at varying intervals after ovulation These observations may be of importance in any consideration of the problem of abortion in women since the natural

mechanisms of oestrous behaviour which ensure mating only near the optimum time in animals do not operate in human beings

## Transplantation Techniques Applied to the Study of the Ageing of the Ovum and Ovary

The method of tissue transplantation has been used for the study of a wide range of biological problems The next part of this paper will discuss how the technique has been applied to the particular problems presented by the ageing ovum and ovary Finally, some of the general hazards that beset the tissue transplanter, the ways of overcoming them, and some experimental results will be described

### The transfer of ova

The technique for the collection of fertilized ova from the Fallopian tubes and their transfer to either the tubes or the uterus of another animal is now well established and has been used especially in the study of reproductive physiology in rabbits and cows, such experiments have not usually, however, been directed towards the study of age changes in the reproductive tract itself The method has also been used by Fekete (1947) to investigate the effect of transferring ova from one strain of mice into an environment provided by another strain of mice Her work shows for example, that the eggs of the two strains, dba and C57, have about equal powers of survival but that the environment provided by one strain is more conducive to survival than that provided by the other Once again, however, the work was not directed towards the study of age change but it indicates the potentialities of the method

### The grafting of whole ovaries

### The onset of puberty

Some of the earliest work using ovarian transplantation was concerned with the factors which influence the onset of puberty It was, indeed, carried out long before an interpretation of the results in terms of pituitary gonadotrophin

could be attempted Foa (1900 1901) transplanted newborn rabbit ovaries into rabbits of different ages One of his experimental groups, in which twelve to eighteen month old rabbits were used as hosts, demonstrated for the first time that transplantation into a mature environment accelerates the maturation of a young ovary This group however, only contained three experiments of which two gave positive results In another group the hosts were five to six years old and the newborn ovary never survived the transplantation Conversely Lipschutz (1925) showed that the transplantation of mature ovaries failed to induce rapid feminization if the host guineapigs were prepubertal Other experiments, which indicate that ovaries of newborn animals develop precociously when grafted into older animals have also been described by Lipschutz (1925) Del Castillo (1929) Engle (1929) and Pfeiffer (1934) Goodman (1934) May (1940) and Dunham, Watts and Adair (1941) showed that immature ovaries which are implanted into the anterior chamber of the eye of adult male and female rats of the same inbred strain also develop more rapidly than normal Œstrous cycles are restored after only a very slightly longer delay (thirteen to seventeen days) than follows the grafting of adult ovaries (seven to fourteen days) In many of these experiments there is some doubt about the final result and Del Castillo for instance believes [illegible]

[illegible] the grafts at autopsy

Hertz and Hisaw (1934) whose work on the sensitivity of young ovaries to gonadotrophin has already been mentioned, grafted four week old ovaries under the renal capsule of twelve to fourteen week old rabbits Ten days later they began treatment with gonadotrophin In four experiments in which the grafts took successfully the hosts ovaries responded normally but the grafts were not affected They interpreted this observation to indicate that the failure of the very young gonad to respond resided specifically in the ovary and was not influenced by conditions in the rest of the intact animal

It is now known, however, that grafts of any age often fail to respond to a dose of gonadotrophin when the host's ovaries remain *in situ*, a finding which is attributed by Lane and Markee (1941) to differential take up of the hormone by the host glands which retain an intact normal blood supply

The anterior pituitary gland of a young animal also develops more rapidly after it has been transferred into an adult host Harris and Jacobsohn (1951) found that pituitary tissue which was transplanted from two day old female rats to the sella turcica of their hypophysectomized mother, very soon functioned like a normal mature gland and restored normal œstrous cycles Harris and Jacobsohn also found that the pituitaries of newborn male rats were just as effective in producing œstrous cycles in the female host

When ovaries are transplanted into adult males, however, only follicular development takes place and no corpora lutea are formed The mature male pituitary apparently fails to secrete the luteinizing hormone which is required for ovulation and the formation of corpora lutea There is, therefore, a functional difference between mature male and female pituitaries By gonad transplantation studies Pfeiffer (1936) was able to show that, at birth, the character of the hormone production of the pituitary is still undetermined and can be directed in one direction or the other by bringing the gland under the influence of male or female gonads Final determination has normally occurred before the age of puberty, after which attempts to change the function of the gland are unsuccessful

## Pituitary transplants and the end of activity of the ageing ovary

Wiesner's preliminary observations (1932) indicate that the ovaries of old rats, unlike those of women, are still capable of responding to gonadotrophic stimuli, which, in ordinary circumstances, the pituitary no longer provides Compensatory hypertrophy after unilateral gonadectomy did not occur in either male or female rats which were over twenty four

months old and which showed evidence of gonadal insufficiency Ovarian function could however be restored by treatment either with extracts of pregnancy urine or with grafts of anterior pituitary tissue Hoffman (1931) also restored œstrous cycles in old anœstrous mice by transplants of calf pituitary tissue It is evident from the illustrations provided by Hoffman that the ovaries of these mice although atrophic still contained many primordial follicles which could respond to gonadotrophin

If these experiments are valid it would seem that while the function of the ovary fails before that of the pituitary in women such is not the case in rodents This inter species difference is further shown by work on the development of ovarian tumours in the ageing ovaries of rodents transplanted into the spleen

## Ageing of the ovary and the development of ovarian tumours

It is known that tumours develop in ovaries that are autotransplanted to the spleen of spayed rats or mice (Biskind and Biskind 1944) The usual explanation for the development of these tumours is that œstrogen from the grafted ovary passes directly into the portal circulation and is destroyed by the liver [illegible]
release of gonadotrop
Severinghaus 1938)
by the pituitary persists and stimulates abnormal growth of the graft While this is true for the rat it may not be so for the rhesus monkey (Van Wagenen and Gardner 1950) [illegible]

[illegible] ases as ovarian function declines at the menopause If there is any reactive tissue remaining in the ovary one would expect ovarian tumours to become more common in older women This expectation is not however borne out by the facts and one must therefore assume either that the level of gonadotrophin is not high

enough or that the ovaries of women have become refractory to stimulation of any sort

In tests on rodents, however, Li and Gardner (1950) found that the ovaries of 340–491 day old mice were just as susceptible to tumour formation as were the ovaries of younger animals Klein (1952) who extended these observations to a group of old mice whose average age was seven hundred and ninety nine days also found that old ovaries may develop tumours but there was some evidence that they were not as sensitive to the stimulus as are young ovaries The positive findings in these experiments can be correlated with the continued presence of active ovarian tissue in such old ovaries and serve to distinguish once again the ageing ovary in women from the ovaries of rodents

## General methodological problems of ovarian transplantation

Much of the earlier work on ovarian transplantation must face the objection that the experiments were not designed in a way that would avoid the difficulties introduced by the immunity reaction which develops when any tissue is homografted One way of overcoming this difficulty is to use what may be called delayed autografting Tissues can be removed from an animal while it is still young preserved for any required length of time by storage at low temperatures and then regrafted into the original donor The results of this form of experimental approach will shortly be discussed by Parkes The method suffers from the inevitable disadvantage that tomorrow s older tissues can never be transferred to yesterday s younger animals It is only possible to graft tissues of their more youthful days into animals that have got older There is also the added disadvantage that the tissue may deteriorate during prolonged storage

An alternative approach depends on the fact that some strains of mice are so inbred that tissues can be transferred from one member of the strain to another without stimulating an immunity reaction Tissues from both parent strains of

mice are also acceptable to the hybrid produced from crossing the two strains In this way it becomes possible to study what happens not only when an old ovary is placed in a young environment and vice versa but also when ovaries of two strains which may differ in the rate in which they become old (see above) are put into a common environment (Cf Huseby and Bittner [1950] who were interested in the influence on the incidence of mammary cancer of ovaries from strains A and Z when grafted into A×Z hybrids )

Many different sites for transplants have been chosen and the functional activity of the grafts has usually been assessed by the reappearance of œstrous cycles and by histological methods Most sites such as the subcutaneous tissues the spleen or the anterior chamber of the eye necessarily preclude the most valuable and for our purpose the essential criterion of function The ovary must be transplanted so that the ova can reach the fallopian tubes become fertilized and implant in the uterus Such orthotopic grafting of ovaries was first carried out in rabbits and guinea pigs which do not have a complete ovarian capsule some fifty years ago Foa (1901) and Castle and Phillips (1913) believed that pregnancies which took place after grafting were derived from the grafted ovary and not from regenerating host ovary Much more recently Whitney (1946) believed that he had successfully exchanged orthotopically the ovaries from a bloodhound to a foxhound although the animals failed to become pregnant afterwards

The fact that its ovary is surrounded by a capsule makes it easier to achieve this purpose in the mouse than in the other two *species that* have been mentioned It is relatively simple to open the capsule remove the animal s own ovary and replace it with another This method of intracapsular graft [illegible] of the [illegible]

Ru [illegible]

dif [illegible] between the genetic and environmental factors which are responsible for the intra uterine death of homozygous yellow mice The Bar Harbor workers,

enough or that the ovaries of women have become refractory to stimulation of any sort

In tests on rodents however, Li and Gardner (1950) found that the ovaries of 340–491 day old mice were just as susceptible to tumour formation as were the ovaries of younger animals Klein (1952) who extended these observations to a group of old mice whose average age was seven hundred and ninety nine days also found that old ovaries may develop tumours but there was some evidence that they were not as sensitive to the stimulus as are young ovaries The positive findings in these experiments can be correlated with the continued presence of active ovarian tissue in such old ovaries and serve to distinguish once again the ageing ovary in women from the ovaries of rodents

## General methodological problems of ovarian transplantation

Much of the earlier work on ovarian transplantation must face the objection that the experiments were not designed in a way that would avoid the difficulties introduced by the immunity reaction which develops when any tissue is homografted One way of overcoming this difficulty is to use what may be called delayed autografting Tissues can be removed from an animal while it is still young preserved for any required length of time by storage at low temperatures and then regrafted into the original donor The results of this form of experimental approach will shortly be discussed by Parkes The method suffers from the inevitable disadvantage that tomorrow's older tissues can never be transferred to yesterday's younger animals It is only possible to graft tissues of their more youthful days into animals that have got older There is also the added disadvantage that the tissue may deteriorate during prolonged storage

An alternative approach depends on the fact that some strains of mice are so inbred that tissues can be transferred from one member of the strain to another without stimulating an immunity reaction Tissues from both parent strains of

of another strain The host animals were necessarily spayed before grafting and conditions for the survival of homografts should have been optimal In 25 experiments the homografts that were recovered at autopsy, between nineteen and forty six days after grafting, had all been destroyed by a homograft reaction in which round cell infiltration and replacement with fibrous tissue were prominent features In some of these experiments œstrous cycles had continued after the operation The explanation for this fact, however lay not in survival of the homograft but in the presence of small masses of ovarian tissue of host origin left behind after the ovariectomy Differentiation between homograft and autogenous ovarian tissue was quite obvious when the material was examined histologically In further experiments the homografts were removed either six or nine days after grafting In all a reaction against the grafts was well under way, in most of them the tissue organisation of the grafts had already been destroyed but a few small follicles and some luteal tissue still looked reasonably healthy The follicular apparatus does not withstand the rigours of homotransplantation as well as the luteal cells, scattered clumps of which may still be seen some time after the rest of the ovary has been destroyed

### The "Potential Immortality" of the ovary

The fact that tissue cultures of fibroblasts can be maintained for very long periods of time is well known The same sort of cellular immortality can be demonstrated for tumour cells by repeated passages of the malignant tissue from one [illegible]

[illegible] achieved Very few attempts have been made to carry out similar experiments with normal organs Loeb (1945) reports the serial transplantation of thyroid glands in Strong A mice The number of serial transplantations ranged from three to seven and the transplants remained in each host for three and a half to six months In some of these experiments the thyroid tissue survived the process of serial

who have developed an ingenious marker gene technique for distinguishing between the offspring from the grafted ovary and from any ovarian tissue that regenerates, have shown that litters can be obtained even from embryonic ovaries that have been transplanted into young adult animals.

Russell and Douglass (1945) have also used the method to study what happens to the ovaries when they are submitted to abnormal hormonal influences during a temporary stay in the spleen of castrated hosts before being returned to a normal endocrine environment. They find that the oocyte producing apparatus is unable to withstand such treatment, although the endocrine function is unaffected.

My own experiments using transplantation methods have been directed along the following lines.

### Problem of technique

The only serious difficulties raised by the technique of intra capsular grafting are incomplete removal of the ovary and bleeding from the cut ovarian vessels. Even the most meticulous surgery cannot always obviate the first difficulty, but the effects can be mitigated by using the gene marker methods introduced by the Bar Harbor workers. Patience seems to be the only way of overcoming the second. The use of a diathermy cautery was unsatisfactory because it damaged the delicate tissues of the fallopian tubes.

The mere fact of transplantation is sufficient, according to Hummel, Fekete and Little (1953) to increase the proportion of ovarian tumours that develop in later life. The results of any work which uses the method may, therefore, have to be interpreted with some caution.

### The survival of ovarian homografts

In view of the widespread differences of opinion about the chances of survival of ovarian homografts, discussed elsewhere (Krohn, 1955), I have carried out a further series of experiments in which ovaries from one strain of mouse have been transplanted orthotopically into the ovarian capsule of mice

This would confirm the general impression that ovarian function in rodents is related to pituitary activity and not to changes inherent in the ovaries themselves

Using CBA and A strain mice for the work, we are also satisfied that a hybrid host can tolerate grafts of ovaries from both parent strains Of the six animals used all have shown oestrous cycles and after mating have had vaginal plugs and become pseudo pregnant So far two of them have become pregnant

Before proceeding any further with the experiments it will be necessary to establish more exactly the rate at which the total number of follicles in the ovaries (both normal and after transplantation) falls off as the mice get older Only when this information is available will one be able to say anything with certainty about the interaction of the ageing environment and the ageing ovary

## Conclusion

It is a far cry from the days of Voronoff and the expectation that transplants of gonadal tissue would serve to rejuvenate the senescent It is now plain that such hopes which rested on the possible benefits of heterotransplantation were bound to be illusory Today even homotransplants of gonadal or of endocrine tissue have no practical clinical value In the future however the method of transplantation may contribute more as a research tool to the problem raised by the ageing of tissues than it ever could have done in the past as a form of treatment

**Acknowledgement**

I am very grateful to Miss F Holt for her assistance in carrying out the experiments

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transplantation and the oldest tissue recovered was forty one months old, which is probably outside the ordinary lifespan of the strain of mice used Loeb (1945) mentions that similar experiments with grafts of ovary failed entirely Some comments by Dunham, Watts and Adair (1941) that are incidental to their main work, also suggest that the ovary does not tolerate being transplanted more than twice

In our own experiments ovaries have been autotransplanted from one side of the body to the other at intervals of two or six weeks Control autografts readily survived a period of up to twelve weeks and gave rise to normal vaginal œstrous cycles In the first group of animals the period between successive transplantations was two weeks After the second transplantation two out of eight animals continued to show definite œstrous cycles and four of the eight showed occasional and irregular periods of œstrus After the third transplantation four out of five animals showed no œstrous cycles at all and only one gave a doubtful positive result The same results were obtained in another group which were continued to a fourth transplantation In the final experiment six weeks were allowed to elapse before the second transplantation Œstrous cycles did not recur during the succeeding six weeks in any of the four animals used The experiments have, therefore, failed in their purpose They are being repeated using orthotopic grafts and allowing a longer interval to elapse between successive graftings

## The behaviour of old and young ovaries in environments of different ages

Combinations of orthotopic grafts have been employed either young ovaries into old hosts old into young, young and old into young, and young and old into old Only Strong A strain mice have been used It is too early to say what the effects on the numbers of oocytes and on reproduction will be, but it would appear that old ovaries grafted into young animals behave more satisfactorily, as far as the data on the œstrous cycles go, than young ovaries grafted into old animals

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of ageing while the donor animal wends its way to senility. The idea of achieving a state of suspended animation at low temperatures is of course very old and depends in its modern form on the reasonable assumption that all vital processes would be suspended at temperatures such as those obtained by the use of dry ice (−79° C) or liquid air (−190° C). Until recently however the act of freezing and thawing was fatal to almost all normal cells of vertebrates, so that little progress was possible in the study of experimental biostasis. This situation was altered radically a few years ago by the observation that glycerol has remarkable properties in protecting living cells against the otherwise fatal effects of freezing to very low temperatures (Polge, Smith and Parkes, 1949).

This discovery has made it possible to keep a variety of living cells at −79° C or −190° C in an apparently stable state for periods of months or years and the work has added greatly to our knowledge of the dynamics of the living cell and in particular of the effects of low temperatures.

## Resistance to Freezing and Thawing

It appears that freezing damage is primarily of two kinds, one due to the hypertonicity of the residual fluid as water freezes out as ice and the other to thermal shock arising from rapid change of temperature either above or below freezing point. The former can be avoided to a large extent by ultra rapid freezing, the latter by slow cooling. In other words it is almost impossible to freeze a normal cell by ordinary methods without subjecting it to one or other type of damage. Glycerol apparently exerts its effect by decreasing the hypertonicity hazard and permitting cooling to be slow enough to avoid thermal shock (Lovelock, 1953).

## Stability at Low Temperatures

The loss of cells on freezing and thawing without storage at the low temperature may be negligible, appreciable, substantial or very large according to the conditions and the

# PRESERVATION OF TISSUE *IN VITRO* FOR THE STUDY OF AGEING

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*National Institute for Medical Research, London*

THE general question of ageing of parts of the body in relation to ageing of the whole can be investigated in various ways. I want to deal with a specialized development of one of them. In the case of organs or tissues which can be grafted it is possible to transfer the organ or the tissue from one animal to another in such a way as to have an old tissue in a young animal or a young tissue in an old animal. Theoretically, as Dr Krohn has indicated, it is thus possible to distinguish between functional breakdown of a tissue due to internal causes and due to failure of its environment. Unfortunately, some of the organs most suitable for homografting and for estimation of functional activity—the endocrine organs other than the pituitary body—are of limited significance in this connection because they are known to be dependent on environmental stimulation and in the case of the gametogenic function of the ovary to be of limited potentiality. Nevertheless, instructive results could be expected, especially where successive passages of the same tissue from old animals to young animals as each host becomes senile makes it possible to secure a big difference between tissue age and body age. All such experiments, however, require that the part shall be subjected to the hazards of homografting. I want now to discuss a recently developed technique which permits of experiments where the part is physiologically *younger than the whole without the hazards of homografting* from a young animal to a contemporaneously older one.

I refer, of course, to the technique of low temperature preservation by which tissue can be held in a state of suspended but potential viability, free from the complications

arises from the failure of biochemical processes to repair biophysical dissolution With the red cell it appears that damage of sub lethal degree occurring during storage may be repaired when the cell is returned to its normal environment It is possible that the general thesis set out by Lovelock [illegible]

the passive dissolution due to biophysical causes

## Isolated Tissues

I want to turn now to the application of this work to the study of the ageing of parts in relation to the whole I have [illegible]

increased on storage and that the cells which survive have not apparently aged Details of technique and data for tissue frozen for up to one year were given at a previous symposium (Smith and Parkes, 1954) By the time similar material had been frozen for two years a number of complications had appeared For one thing, several of the donor rats died during the second year For another, several known failures in the maintenance of the flasks of liquid air had occurred, and tissue known to have warmed up had been discarded As a result of these circumstances there was available after approximately two years a number of old ovariectomized females without corresponding stored ovaries as well as stored ovaries without corresponding old animals An experiment was therefore designed in which in addition to the autografting of long stored ovaries, fresh ovarian tissue from young females was homografted into old ovariectomized females and long stored ovaries into newly ovariectomized young females (Table I) The results of the first experiment suggested that the tissue autografted after storage for approximately two years (line one of the table) did not take well

type of cell On storage at the low temperature the cell may be apparently stable, as with bull spermatozoa (Rowson and Polge, 1953) and rat ovarian tissue (Parkes and Smith 1958), or the loss caused by the initial freezing may be increased, as with fowl spermatozoa or human red cells according to the type of cell, the medium and the temperature In any case, cells that survive storage have apparently not aged Frozen bull spermatozoa, for instance, after thawing, survive at normal temperatures as well as do fresh sperm

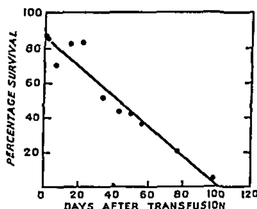



FIG 1 Survival after transfusion of red cells stored at —79° C for six months and at +4° C for eleven days Survival is normal (Mollison Sloviter and Chaplin *Lancet* 1952 2 501 By permission of the *Lancet* )

and human red cells, after long periods of storage, have a normal expectation of life in the circulation after transfusion (Mollison, Sloviter and Chaplin, 1952)

A significant deduction is to be drawn from these observations It may be assumed that biochemical changes are arrested at the very low temperatures employed The changes taking place in cells which suffer damage on storage must therefore be physical ones occurring in the absence of the usual biochemical processes of repair The various factors involved in the case of the red blood cell have been investigated in detail by Lovelock (1954) according to whom the damage caused by storage under unfavourable conditions

arises from the failure of biochemical processes to repair biophysical dissolution With the red cell it appears that damage of sub lethal degree occurring during storage may be repaired when the cell is returned to its normal environ ment It is possible that the general thesis set out by Lovelock is of more general applicability to the exhaustion and repair of cells it might perhaps be possible to regard ageing as the result of increasing failure to repair by active biosynthesis the passive dissolution due to biophysical causes

## Isolated Tissues

I want to turn now to the application of this work to the study of the ageing of parts in relation to the whole I have already indicated that ovarian tissue of rats can be preserved for long periods in liquid air at $-190°$ C that there is some loss of cells on freezing and thawing but that the loss is not increased on storage and that the cells which survive have not apparently aged Details of technique and data for tissue frozen for up to one year were given at a previous symposium (Smith and Parkes 1954) By the time similar material had been frozen for two years a number of complica tions had appeared For one thing several of the donor rats died during the second year For another several known failures in the maintenance of the flasks of liquid air had occurred and tissue known to have warmed up had been discarded As a result of these circumstances there was avail able after approximately two years a number of old ovariecto mized fem

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... aries into newly ovariectomized young females (Table I) The results of the first experiment sug gested that the tissue autografted after storage for approxi mately two years (line one of the table) did not take well

The second experiment (line two of the table) seemed conclusive on the point The homografting of long stored ovaries into newly ovariectomized young females (line three of the table) and the reciprocal experiment of homografting fresh tissue from young females into the old ovariectomized

**Table I**

GRAFTING OF LONG FROZEN OVARIAN TISSUE INTO ORIGINAL DONOR OR YOUNG OVARIECTOMIZED FEMALES AND OF FRESH OVARIAN TISSUE FROM YOUNG FEMALES INTO LONG OVARIECTOMIZED OLD FEMALES

(Donors all young females approx 100 g B Wt )

| | [illegible] | Time ... (days) [illegible] | Recipient (all ovariectomized) [illegible] | [illegible] | [illegible] | Period of observation after grafting (days) [illegible] | Total No. of [illegible] |
|---|---|---|---|---|---|---|---|
| 1 | Autograft | 674 47 | [illegible] | 3 | 18 | 5[illegible] | 13 |
| | Autograft | 1 | [illegible] | 1 13 | 12 | 3 | — |
| 3 | Homograft | [illegible] 7 | Young females | 4 3 | 1 | 3 5 | 11 |
| 4 | Homograft | Fresh [illegible] | Old females [illegible] after ovariectomy | 0 | 0 | 5 | 37 |
| 5 | Homograft (control) | Fresh tissue | Different young females | 9 10 | 1[illegible] | 28 | |

females (line four of the table) showed clearly that the long stored tissue not the old females was at fault It must be assumed therefore that there had been an undetected breakdown in maintenance of the liquid air flasks or that the tissue had deteriorated spontaneously during its second year of storage It has not yet been possible to distinguish between these alternatives

## Isolated Organs

In some ways the possibility of long term preservation of isolated organs is of even greater interest than that of cells or tissues An approach has been made to this problem by

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Fig. 4. Homograft
follicle and normal
grafted to old rat
nine days later and
52 [illegible] transplantation (× [illegible])

work on the isolated uterus of the guinea pig, survival after freezing in various media for various times being assessed by its spontaneous contractions and its responsiveness to histamine Conditions for complete survival have not yet been determined, but a considerable degree of reactivation is compatible with freezing by present methods, and the damage is not increased by storage (Parkes and Smith, 1954) It is likely that present methods with the isolated uterus can be much improved and applied to other organs, so that a




FIG 5 Contractility of isolated uterus of guinea pig after storage for three months at −79° C in 20 per cent glycerol Ringer Figures indicate μg of histamine added to bath Contractility is subnormal but still vigorous

great extension of experiments involving variation in the age of the part in relation to the age of the whole can be envisaged

## The Whole Body

The work outlined above naturally turned our attention to the problem of cooling the whole animal to such an extent as to arrest all vital processes and confer upon it, in a state of suspended animation, the kind of comparative immortality which can now be effected with bull spermatozoa and other cells appropriately frozen and maintained at −79° C

Any such project obviously presented enormous difficulties, particularly in view of the different requirements for safe freezing of different types of cell and of the overriding neces

sity for a comparatively high concentration of glycerol or other neutral solute for the freezing of individual cells and tissues An approach to the problem has, however, been made possible by the work of Andjus (1951) who evolved a method by which rats could be cooled to deep body temperatures approaching 0° C and revived again after cessation of heart beat and respiration for one or two hours (Andjus and Smith, 1954) Application of this technique to hamsters chosen because of their known adaptability to body temperatures in the range between 2 5° C and 38°C, enabled work to be concentrated on the effects of reducing body temperature below zero (Smith Lovelock and Parkes 1954) Intriguing results were obtained As the deep body temperature goes below zero one or other of two things happens (a) Some hamsters start to freeze peripherally and the deep body temperature stabilises at about −0 6° C when the surrounding bath is held at −5°C to −7°C Crystallisation of body water proceeds slowly but steadily and the extremities and superficial tissues become hard frozen Complete recoveries have so far been effected after periods of freezing up to one hour, by which time it can be calculated from the rate of heat loss that not less than 15 per cent of the body water has crystallized (b) Other hamsters start to super cool as the deep body temperature goes below zero, and the temperature falls steadily until it approaches that of the bath Deep body temperatures as low as —5°C without crystallization have been recorded, and such animals can be resuscitated completely More often crystallization takes place suddenly from the super cooled state, accompanied by an abrupt rise of temperature to about −0 6°C Crystallisation arising in this manner appears to be diffuse throughout the body and is more damaging than that arising peripherally from gradual freezing

These experiments though highly intriguing touch merely the fringe of this subject, and it must be emphasized that there is no immediate prospect of retaining viability in whole animals frozen to very low temperatures

## Conclusion

The work on the preservation of cells, tissues and organs at low temperatures offers immediate possibilities of work on the comparative ageing of different parts of the body It opens up the ultimate possibility of inducing suspended animation of the whole body for indefinite periods, i e the defeat of ageing as it is now understood Whether this latter prospect comes within the realms of gerontology, or whether it is in itself desirable, I do not know It seems, however, that the idea of biostasis of the whole body, macabre at first sight, holds nothing new in principle, being woven into the fabric of countless human beliefs, legends, and stories, from the resurrection of the dead to the awakening of the Sleeping Beauty

**Acknowledgement**

The hitherto unpublished work on ovarian grafts recorded above was started in collaboration with my colleague Dr Audrey U Smith, to whom I am also indebted for Fig. 5

The operative procedures for the grafting experiments were carried out by Miss J Masson and Miss M Mansi

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### DISCUSSION

*Medawar* Parkes and Krohn between them have not quite covered all the techniques one might use to construct "age chimeras", that is

animals with tissues of different ages For completeness' sake, I think one should add parabiosis, which Prof McCay mentioned yesterday

I'd like to show you a concrete example of the use of what Krohn described as "delayed autografting". Billingham and I have been [illegible] purpose The principle of [illegible] the removal of tissue from [illegible] to the same animal when [illegible] ared on the chest wall of a [illegible] s four grafts on it, which have been in position for eight days As you see, two of the grafts are pale, and two of them are rather black The two darker grafts were removed immediately the animal was born and stored at the temperature of dry ice after soaking in glycerol Ringer The two whiter grafts were removed only one day before the grafting operation They were soaked in glycerol Ringer, frozen to the temperature of dry ice for twenty-four hours, and then thawed out again The four grafts were then transplanted simultaneously One can see from the picture that the four grafts have survived and are surrounded by about the same amount of new epithelial outgrowth (Outside the raw area are two "fitted" grafts, one young and the other of the same age as the host these have healed perfectly but are not, of course, surrounded by outgrowth ) Billingham and I started quite a number of experiments of this type, but we are now running into a difficulty which Parkes has called [illegible] ost period which [illegible] hundre [illegible] experi [illegible] young [illegible] degree of deterioration on storage at all At seven hundred days and still more so in a very recent experiment with grafting new born skin [illegible] tial promise, I am afraid we may be severely handicapped by the fact that one does, as Parkes has pointed out, get progressive deterioration on storage

*Parkes* I think what is wanted is some more work We have to deal with one problem after another as it comes up Four years ago we couldn't preserve any of the cells in which we were interested for any worth while length of time Gradually the conditions required for each different kind of cell or tissue, conditions which may be totally different have been worked out Unless you have already tried a large range of media and temperatures and methods of freezing and thawing, it is premature to say that the skin won't last at a low temperature

*Medawar* You think that for each tissue it will be possible to find a medium in which biophysical deterioration on storage won't occur? Are you prepared to go on record as saying that?

*Parkes* I'm prepared to go on record as saying that I have no doubt



Fig. 1 (Medawar)

[To face page 1 0

hypotonic media to reduce the residual salt effect, has now enabled the loss to be reduced to 1 per cent over a year or more.

*Leeds*: I think you can get information on that point from the food industry. If one stores fish, say herring, at –20° they are edible; if one stores them at –80 or so they become inedible. The conservation of proteins seems to depend on the temperature at which one stores

... of course there's no reason to suppose the offspring wouldn't be normal. Tens of thousands of calves have now been pro...

*Hol...*: Yes.

*Comfort*: If one had to work with the rabbit or dog, I wonder whether artificial parthenogenesis would give you a source of material.

*Coudry* What kind of physical changes continue in these tissues Dr Parkes?

*Parkes* Analyses of the media, in the case of the red cells, suggest that there has been loss of lipids and ... But for details I'm afraid I h...

*Coudry* It seems to r... place would almost necessarily...

*Parkes* Well, chemica...

...ge exist

*Krohn* That is an old subject, which has been reviewed again and again My personal view is that the organism is endowed with so many ova and does not go on making fresh oocytes I do not think any of the experiments which purport to demonstrate ... showing ... are at ... explan... epitheli... that the v... If the germ... it must inc... Another m...

... I believe that any such replenishment probably does not happen, and that there is this constant ... over...

ov...

wo...

*Krohn* Certainly That is a point, the importance of which I hope I made clear in my paper

*Parkes* I ... piece] which ... Karl Pearso...

# RESEARCH AREAS IN GERONTOLOGY NUTRITION THAT ARE NOW NEGLECTED

C. M. McCay, Ph D

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The subject chosen for discussion today recalls an experience of ten years ago Our community in rural New York State serves as host each summer to more than a hundred children from the slums of New York City. For five years, we had as our guests a starved, backward boy and his younger sister When this boy was about twelve years old, I discovered he could not tell time nor read well. I started him reading a story book and suggested that during the afternoon he should underline every word he did not understand so that I could explain the words on the following morning. Much to my surprise, the next morning I found that with the exception of "the", "a", and "is" all words had been carefully underlined

After labouring since 1927 in this area that attempts to relate the food eaten to conditions observed in the body during the latter half of the life span, it has become clearer each year that most of the words in this field must be underlined as unknown Perhaps, this is merely the ever growing pessimism of an ageing person

In considering research areas for profitable study in relating nutrition to ageing it may be worth considering first the species of animal life that can be studied most profitably and secondly the disciplines of a scientist that seem most suited for the attack.

Unfortunately, those of us who were transformed painfully into biochemists just prior to World War I through the channels of classical chemistry, physics and mathematics were left with substantial prejudices if not contempt for such

*Cowdry* What kind of physical changes continue in these tissues, Dr Parkes?

*Parkes* Analyses of the media, in the case of the red cells, suggest that there has been loss of lipids and lipoproteins from the cell But for details I'm afraid I have to refer you to Dr Lovelock

*Cowdry* It seems to me that any biophysical change taking place would almost necessarily be followed by a chemical change

*Parkes* Well, chemical change must take place very slowly in a medium which is completely frozen up.

*Cowdry* Dr Krohn, have you any special ideas about the value of the two views concerning oogenesis?

*Krohn* That is an old subject, which has been reviewed again and

[illegible]

epithelium is maximal around the time of ovulation. It is at this time that the volume of the ovary and therefore its surface area is greatest. If the germinal epithelium is to act as an epithelium and cover the ovary, it must increase in extent and its component cells will have to divide Another important point to emphasize is that whenever the total number of oocytes in the ovaries of any species has been counted, the results

[illegible]

does not happen, and that there is this constant wearing away of the

[illegible] h) the

[illegible] young

woman

*Krohn* Certainly That is a point, the importance of which I hope I

[illegible]

In gerontology this analogous thinking in relation to nutrition may indicate that much can be learned by use of single celled organisms or very complex insects but that generalizations must have some anchorage in experiments with man himself

Bacteriologists appreciate that cultures grow old but limited studies such as those of Otto Rahn (1951) indicate that the process is fully as complicated and confusing as appears the ageing of higher vertebrates The use of insects seems to have promise not only because of the vast numbers with which one can work cheaply but also many species pass through their life cycle during brief periods of days or weeks In his discussion of longevity in the animal kingdom Metchnikoff (1908) noted Between these extremes of long and short life there is found amongst insects almost every gradation of longevity

One need not review the world of fish and lower vertebrates to appreciate the value of studying any of the numerous chordates, some of which have a reputation true or false for great longevity

One can certainly conclude that no life will be wasted if devoted to the study of ageing material of any form whether it be the seeds of plants or man himself

To the student of ageing moulds or rotifers the specialist devoting his life to the study of man himself may quote Goethe with genuine truth Wir sehen uns wieder weit gar weit *von hier* —gerontologists will ultimately *meet upon* common grounds

For the remainder of this discussion it may be well to draw upon our own *experiences* of twenty seven years in order to note the fields that might have been cultivated profitably and that were *neglected* For a substantial fraction of this period our laboratory used three widely different classes of animals for research upon growth and ageing These included several insect species but most of the research was done upon the small cockroach *Blatella germanica* For fifteen years studies were in progress at different times upon species of

fields as physiology, bacteriology, zoology, and entomology. Only during the last quarter of a century even in the field of nutrition has this climate of attitude changed.

Respect for comparative biochemistry and nutrition first came from agricultural science in which men were accustomed to thinking of the common species of domestic animals. Since the early nineteenth century and the gelatin experiments of Magendie the science of nutrition from medical schools has rested heavily upon the dog and to a lesser extent upon the mouse, rabbit and rat, towards the close of the nineteenth century.

However, only during the past thirty years has the biochemist who works upon bacteria become respectable and the one who devotes his love to insects or to any except a selected few of accepted vertebrates is a subject for humour and contempt if not suspicion. I have never forgotten the peculiar expressions on the faces of my friends on both sides of the Atlantic when they used to come to watch me weighing my cockroaches, or my trout in the fish hatchery. Fish have now become accepted fodder for the mill of the nutritionists but insects are still not taken seriously as a form of life whose biochemistry or gerontology may be worthy of study.

If one indulges in some retrospect in regard to the discovery and isolation of the vitamins, one must wonder how much more rapid might have been the progress if biochemists had neglected their mice and rats and given all their devotion to micro-organisms and insects (McCay, 1953). In the case of the water soluble vitamins substantial progress would have been assured, but for the fat soluble vitamins there might have been little progress. No insect has been discovered thus far that needs either vitamin A or D, although there may be many insects that need these factors and have never been studied. If man had rested his nutritional science upon insects we would probably be reading in the modern works about human nutrition that each of us should consume so many grams of cholesterol daily because this does seem to be an essential for insects.

because goldfish have long been pets, are easily cared for and cheaply fed upon dry feed mixtures. Quite the contrary is true for the various trout species with which we laboured for so many years. They require special water conditions, unique diets and are somewhat difficult to culture.

Turtles, whose hearts we have all admired since our earliest study of physiology, are deserving of the most intense study. In spite of our admiration for their tough hearts little is known about the biochemistry involved. Turtles can be hatched and reared in confinement. Two decades ago in a study that was never published, my associate J. B. Sumner and I injected crystalline urease into the jugular of a snapping turtle. Many times the amount was used that would kill another species such as a rabbit but no level was found that even annoyed the turtle. Later, we found the blood of the turtle to be naturally quite rich in ammonia. Turtles, undoubtedly, know biochemical secrets about ageing that might be useful to man.

Finally, domesticated birds such as pigeons, chickens, turkeys, geese and ducks offer special opportunities. Long time study of any of these species would undoubtedly lead to ad… and facts t… husbandry F… consideration of the span of life and old age diseases little attention has ever been given to any of these species.

As we look back over a quarter of a century, we believe if we could have foretold the progress that was to be made in the study of the genetics of the mouse that we would have selected this species in preference to the albino rat. One of the earliest demonstrations of the life span of a rodent was made in a study of mice by Robertson and Ray (1920). Likewise the very important observation that tumours could not be implanted in partly starved mice was made by a student of Ehrlich early in this century (Moreschi, 1909).

Many thousands of mice are wasted each year in genetic laboratories because the breeding animals are usually only kept until early middle age. The great disadvantages of mice from

trout, with limited research upon carp and northern pike During the whole of the past quarter of a century, the white rat has been subject to continuous study

Each of these three classes which we used responded similarly to the most important factor that we have ever discovered in extending the span of life Each can be maintained for long periods upon diets adequate in quality but inadequate in amount After long periods of retarded growth each can respond to increased food allowance with resumption of growth This means that these growth relationships which are known to have a greater influence than any other upon the total span of life and the terminal, chronic diseases of the laboratory rat are subject to study using either insects or fish Part of the secrets of ageing may certainly be unravelled by the use of these lower species which can often be studied in larger numbers and at much less cost than white rats

Furthermore, evidence is slowly accumulating in three different nations that the slow growth of dairy calves is reflected in superior life time performance of the mature animals This extends the work relating growth and ageing still further because it involves a ruminant that has superb synthetic powers in providing its own needs for water soluble vitamins and essential amino acids

The use of insects by the biochemist offers unique opportunities because the cost of such research is modest Such studies would seem to be especially worth while for scientists who have retired upon modest pensions but retain their faculties for study and research This would seem to be a natural gerontological research opportunity for practising gerontologists because such study makes modest requirements for space and equipment The chief hurdle might be the wife of the researcher who would probably not appreciate making pets out of pests

Among lower vertebrates, three promising areas have been almost entirely neglected Some species of fish such as those of the carp family afford especially unique opportunities

when the rat is a young adult about six months old. Most attempts to keep rats free from this disease by moistening food, spraying the air with antiseptics, having people wear masks in the rat room and the feeding of antibiotics have failed. The feeding of antibiotics does not even protect the teeth of the rats from decay. Some success has been claimed against this lung disease if young are transferred to healthy mothers at the time of birth but no evidence has been provided that such rats can escape the disease during the latter half which is the second year of life (Nelson, 1953).

Rats retarded in growth when fed small amounts of high quality diets delay the onset of this disease until control animals have died with it under conditions that are commonly termed old age for rats. This has made one pathologist suggest that only those who have studied rats subjected to retarded growth have ever seen rats with normal spans of life. This might suggest that all experiments that have not involved growth retardation have merely dealt with foreshortened life-span.

This introduces the problem of misleading titles of articles

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the point of view of the biochemist and nutritionist is that they are very subject to epidemic diseases and difficult to use in any type of chemical balance work since they have the habit of scattering feed badly

Mice have the same advantage as rats and hamsters in passing from birth to old age within a period of about two years In recent years the studies of M Visscher and associates (unpublished) have indicated that mice are very sensitive inasmuch as small modifications of diet seem to change markedly the terminal diseases of old age

White rats have served the nutrition laboratories for about seventy five years Hence, their nutritional requirements are better defined than most of those of the common species In spite of the long use of rats their genetics has been little studied Some breeding studies have indicated that strains can be selected which differ very much in efficiency of feed conversion during growth Some strains are also much more subject to tooth decay than others But compared to mice the rat is an unknown in genetics

The assets of a rat in terms of a two year span of life and ease of feeding for chemical balance studies have already been indicated Furthermore modest knowledge has accumulated in regard to the pathology of this species in old age

Since the white rat has been used more than any other species for life span studies it may be well to scrutinize the weaknesses in such research This has become more important in modern times because public interest has stimulated the writing of an ever growing volume of literature based upon a meagre background of experimental science Opportunists among writers have sensed easy profits from such writing due to the large number of older people and the increased interest by most of them in their own well being Such popular writers neither desire nor are able to evaluate critically much of the meagre evidence from research laboratories

The greatest weakness of the white rat as it ages is the development of the chronic respiratory disease commonly called "bronchiectasis" This can usually be detected easily

when the rat is a young adult about six months old. Most attempts to keep rats free from this disease by moistening food, spraying the air with antiseptics, having people wear masks in the rat room and the feeding of antibiotics have failed. The feeding of antibiotics does not even protect the teeth of the rats from decay. Some success has been claimed against this lung disease if young are transferred to healthy mothers at the time of birth but no evidence has been provided that such rats can escape the disease during the latter half which is the second year of life (Nelson, 1953).

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determine if the young born late in the life of a mother might produce young with a longer potential life No results have come from this study after several years but a few rat mothers have reproduced when past the age of two years

If one turns from the rat to examine some of the other common laboratory animals such as the Syrian hamster the rabbit and the guinea pig one finds that these have some assets but probably less use than the rat The hamster would seem the best since it grows old at about the same rate as the white rat Two studies with this species have indicated that they have a mean life span of about three months longer than the white rat Furthermore the male seems to live longer than the female of this species although this may have been some accident in relation to the stress of the female of this species when it has young (McCay, 1949)

The rabbit has not been used for the study of ageing because of its long span of life and poorly defined nutrition The habit of the rabbit in eating its night excreta makes it difficult to study in the nutrition laboratory since animals must be either collared or the complications from the ingestion of products created in the intestine be faced *Long ago*, Metchnikoff maligned the large intestine and considered it a liability in relation to life span When the waves of science turn their interest towards the large intestine again the rabbit may be the animal of choice since it both creates nutrients within this organ and carries on a substantial absorption from it

Both the rabbit and the guinea pig have rather too long spans of life to interest those who must hurry to solve the problems of old age Furthermore both of these species are subject to epidemic diseases With the potential use of newer marker substances such as chromic oxide and titanium oxide these species may prove especially valuable in studies of intestinal absorption The rabbit seems unique because it maintains a much greater acidity during gastric digestion than do other species such as the dog, and because the rabbit can adapt itself to a wider range of foodstuffs than even the rat

money they are seldom repeated Our own experience has made us very dubious about the adequacy of conclusions based upon a single study even when these are certified by the statistician This is the reason we repeated our study of retarded growth three times in the course of about fifteen years

In spite of these weaknesses of the rat for research upon old age it has great merit The female of this species outlives the male as she does *in the case of man* so this great basic problem of so much interest to the sociologist and economist is subject to study using the white rat The great problem of *extension of life span by the retardation of growth is easily* studied with the rat Only a beginning has been made in determining why growth retardation should affect chronic diseases and even the development of malignant growth Unfortunately, in the whole field of cancer research no one has discovered a closer relationship between nutrition and malignancy than this rather indirect one in which partial fasting inhibits the onset or growth of a tumour Either mice or rats are useful in this area of investigation (Visscher *et al*, 1942)

In some respects white rats afford rather unique opportunities They lend themselves readily to parabiotic unions in which two individuals resemble externally Siamese twins and share in common many substances exchanged through the humoral fluids such as potassium iodide, lactose, strychnine, methylene blue thyroxine insulin and sex hormones Since little is known about the ageing of such pairs of animals or even the span of life we prepared a couple of pairs in the summer of 1953 and they have survived about a year No one has reported such operations to unite young and old

Rats have been extensively used in psychology research but very little has been done in relation to age Likewise, in studying the effect of age of the mother upon the quality of progeny, the rat affords unique possibilities In our own laboratory, after observing some years ago that the long lived rats came from a few mothers, a study was made to

themselves Among the 13,000,000 older people in the United States, only a negligible fraction are contributing to studies of their own welfare There are a few bright spots of old age research such as the Baltimore City Hospital and Nathan Shock's group or the Moose Lodge in Orange Park, Florida

Under the New York Department of Mental Hygiene are more than 30,000 older people being given little more than custodial care One attempt was made several years ago to assemble 900 of the undisturbed older patients in one hospital to serve as a research centre, but this had to end when the hospital was taken over by the air force and the commissioner who had such extensive vision was replaced in the administration

Furthermore, under departments of mental hygiene are thousands of feeble minded patients who are destined to remain wards of the state for the whole of their lives Most of these could very well be participants in studies of ways to improve their own well being even if they could not appreciate what they were doing

An example of a crude but profitable experiment that we did some years ago with the spastic feeble minded may be worthwhile The commissioner at that time asked us to see if we could develop some mush type food for the spastic children because so much time was taken in feeding them the institutional diet As I observed the feeding of these bed-ridden patients I came to realize they were getting a very poor diet because the attendants found it easy to give mashed potatoes, macaroni and carbohydrates but difficult to feed vegetables *and meat* The salvation from bad malnutrition in this case was the daily allowance of whole milk

After making a number of test mixes we found that one containing cooked potato flour, whole wheat biscuits, dry skim milk, wheat germ, dried egg, brewer's yeast, margarine with a small amount of alfalfa meal and salt proved very satisfactory *This* proved simple to prepare with hot water and easy to feed No careful study could be made of these

Among the larger species of domestic animals, the dog the sheep and the monkey all have merit Thousands of very old dogs are killed each year in kennels because they have become too old for breeding purposes Part of these are available for research laboratories, especially in fields such as nutrition where the animals may not be mutilated For some years we have had a supply of such dogs given us We have accepted any registered purebred dog that exceeded eight years of age In some cases such as dogs from kennels no conditions are attached to the gifts In the case of pets we have abided by any restrictions placed by the giver except we have reserved the right to put the dog to sleep in case it was suffering from an incurable disease

Although the dog is one of the most difficult species to study in nutrition because of such factors as emotional attachments and reactions to environment, it may prove the most useful for old age studies because it is large enough to obtain blood samples and because of the extensive knowledge built up during several centuries in regard to the physiology of the dog

Sheep have been little used in the study of ageing but are available in large numbers at modest prices Old ewes when they are sent to the slaughter house to be rejuvenated into lamb for the consumer are worth very little and afford inexpensive research material

Some discussion has been given to the use of monkeys for old age research This use was debated in Washington during several years when the colony of monkeys on the island off Puerto Rico was being abandoned by the universities that established it No progress was made in regard to this use of the colony One American university has toyed with the idea of establishing a monkey colony within their physiology division for old age research This would be very expensive and would require a plan of operation under a group of scientists that would be continuous since the monkeys might outlive their masters

The most neglected of opportunities are found among men

older recipients of welfare if for no other reason than that a healthy person is cheaper for the community to maintain than a sick one

Very little has been or is being done in this field It takes a team of a visionary philanthropist and an imaginative nutritionist Co-operation with American welfare departments would not be difficult

In conclusion, it looks as if the most profitable team for attacking the basic problems of animal gerontology consists of the nutritionist and pathologist In the field concerned with people the best team might be the nutritionist and the well trained physician

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children in comparison with the control group continued upon the institutional diet but from appearances they seemed to develop better colour and better condition of the hair Recently, the dietician told us that she believed they are seeing fewer attacks in a group of children fed this diet and subject to epileptic type of seizures This makes one wonder if a complete diet according to modern nutritional knowledge may yield results similar to those found during the past year when a deficient infant food was supplemented with pyridoxine

In many fields we could learn how to improve human well being by having those trained in research work in this manner with the patients Much of such work could be done in part by time contributed either by retired or active researchers

The hindrances to such programmes are lack of under standing of research by the public The common impression even among scientists, is that there is usually one group in a research programme that is injured Such need not be the case if one studies some current practice compared with a supplemented one

Most mental hospitals are administered by psychiatrists who have a very limited interest or understanding of physical research This restricts progress in the whole field of mental disease and is partly responsible for the philosophy of building more and more mental hospitals instead of spending more efforts in the field of prevention and cure The day may come when the psychiatrist will work side by side with other specialists inside and outside the mental hospitals instead of functioning as now like a semi blind feudal overlord

Another area that affords great opportunities for research with people is in our prisons Long ago, the biochemist, Lohn, called attention to our neglect of our life term prisoners He felt that participation in a research venture might add much to the lives of many prisoners because it is the only way they could contribute something to the world around them As far as we are aware no progress has been made in this field

Finally, there is great need for continuous study of the

1953) or carbohydrate or even occasionally substances like hæmatochrome (Droop, 1954). Too much food of the reserve type, however, may *per se* limit nuclear growth and reproduction The unicellular green plant *Hæmatococcus pluvialis* forms cysts when the supply of nitrogen is too small to support growth The cysts remain small and green except in the presence of light or acetate when they become large and coloured due to the accumulation of fat and hæmatochrome. The fat cysts germinate much less readily than the small green ones *Tokophrya infusionum* again (a suctorian protozoon) can be fed exclusively on *Tetrahymena geleii* and can be grown with it as the sole source of nutriment (Rudzinska, 1953) *Tokophrya* differs from most protozoa in that each cell has a limited

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*phrya* for their energy expenditure, cytoplasmic and nuclear growth, and cell division, all goes well. If, however, the numbers of *Tetrahymena* are unlimited the supply of readily oxidisable food outstrips the requirements of *Tokophrya* for growth and energy expenditure and the cells begin to lay down large amounts of storage material After a time they cease to reproduce in the normal way, turn into giant cells and die before their normal life-span has been completed. Lilly (1953) has found that the formation of giants can be prevented by adding a mixture of guanylic, adenylic, and cytidylic acids and of uracil (in other words of nuclear material) to the medium and it would appear that the suctorian, which has no control over its appetite, cannot obtain enough nuclear material from its standard diet to enable it to synthesize proteins and enzymes (Gale and Folkes, 1954) as rapidly as it can build up reserve materials in the cytoplasm.

Thus, when unicellular organisms become overnourished, nuclear growth and division may slow down. This will *per se*

# A FANTASY ON AGEING AND THE BEARING OF NUTRITION UPON IT

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The plane of nutrition has a profound effect on growth, reproduction and the expectation of life. This is true not only of complex organisms but also of protozoa; it may be true of all cells.

Actively growing rats, pigs and other animals have some innate mechanism which adjusts their intake of food, and consequently of calories, to their expenditure of energy. If *the diet at this level of intake does not contain enough* protein to satisfy their full demands for growth, the animals do not grow at the maximum rate of which they are capable—but they do not get fat. In an organism, however, without this control over its 'appetite', anything which limits growth without interfering with the caloric intake leads to an accumulation of storage material, ultimately in great excess. The protozoa *Tetrahymena*, for example, can be grown in a synthetic medium, and with Dr Hamilton's help we have succeeded in producing giant cells full of fat, by giving a diet which provided plenty of calories but not enough protein to satisfy the capacity for protoplasmic growth and cell multiplication (McCance, 1953). We have also produced giants on the normal—full protein—diets by stopping the multiplication of the organisms with nitrogen mustard. One can play a similar trick on unicellular green plants and make them very obese by providing them with the facilities for photosynthesis or for laying down reserve materials but no source of nitrogen and inorganic salts. The material stored is more variable in the green plants and may be fat (Fogg

It has been reported that undernutrition in some of the flat worms leads both to a reduction in size and weight and also to the reappearance of immature features—in other words to rejuvenation There is no evidence that undernutrition can do anything like this to the higher animals but it certainly can so delay the metabolic processes that by it the normal life span of an animal can be greatly prolonged A start has been made (Brody, 1945, McCay, 1952, Hammond, 1952) but there is still a great deal of interesting work to be done on this subject There is as yet no indication that any of the higher animals can be made immortal in the sense that many protozoa may be so regarded Specialization and integration have been the cause of this 'mortality" but once a cell, even of a highly specialized organism, has been removed from its surroundings and isolated in tissue culture the system may become immortal and heart muscle may be kept alive and beating indefinitely provided the cells are able to grow and reproduce themselves

If undernutrition can so delay the metabolic processes that it can prolong the whole life span of an animal, overnutrition can certainly do the reverse It may not be easy to over nourish a normal animal during growth if the ideal food for growth is provided for it, and our big pig is a good example of this, but the fate of the mouse which has been overnourished (Silberberg and Silberberg, 1954) or of the rat whose hypo thalamus has been punctured (Kennedy, 1950, 1951, 1952) is a proof of what may happen provided something can be done to break down the mechanism which normally adjusts food intake to food requirements, and the obese child who has over-eaten for psychological reasons may be considered as the human counterpart of these animals Once an organ [illegible]

[illegible] doubt about the effects of over eating on the expectation of human life

It is clear now that "efficiency" at all ages depends upon being in the right plane of nutrition In nature the time for

accentuate the overnutrition and the cells may become very large, nuclear growth and division then cease and the cells die

The higher organisms differ from the protozoa in that they all have some innate mechanism of control which adjusts their food intake to their requirements In them as in *Tokophrya* but not in most protozoa, growth is limited and

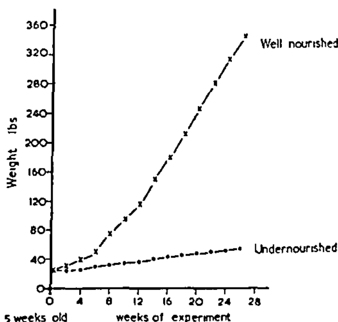



FIG 1 Growth of two pigs one well nourished the other undernourished

life is limited, but both can be modified by nutrition Fig 1 shows the growth curve of two pigs, littermates one had been fed on unlimited amounts of a first class diet for growth purposes and the other had had exactly the same diet, very severely curtailed It is known from the work on rats (McCay, *Maynard, Sperling and Barnes*, 1939, *Brody*, 1945, McCay, 1952) that this small pig might have been kept like this possibly even for years, while its littermate lived out its normal life and died Whether it would then have grown and matured normally as the rat does remains to be discovered

for growth In the early stages the calorie intake may be reasonable and although the child does not grow the liver cells fill with fat and the liver itself becomes enormous Judging by what happens in protozoa one would expect that the expectation of life of each of these liver cells would be curtailed and also its powers of regeneration The cells certainly disappear before their time and are replaced with fibrous tissue If the child survives kwashiorkor in his early days he often has a small cirrhotic liver in later life Alcohol again may not be the *toxin* which kills the liver cell of the heavy drinker so much as the food which overnourishes it It is highly suggestive in this connection and reminiscent of *Tokophrya* that the nucleic acids should accelerate the process of regeneration in liver cells (Newman and Grossman 1951) The pancreatic islets of the obese may not die from over work but from overnutrition Why overnutrition should hasten the death of a cell has not yet been established but it is reasonable to consider it with the apparently opposite problem of why undernutrition should prolong its life Starvation has been found to raise the nuclear/cytoplasmic ratio of the liver cell of the adult rat Marion Harrison (1953) one of our collaborators found that starvation reduced the cytoplasm of the liver cell and to some extent the ribonucleic acid within it but not the deoxynucleic acid of the nucleus itself (see also Davidson and Waymouth 1944) The cells in an organ which is undergoing active growth and hypertrophy are also small and have a large nuclear/cytoplasmic ratio Overnutrition increases the reserve materials within all animals whether unicellular or multicellular and although the segregation of the storage materials within specialized cells in the higher organisms may protect other also specialized cells from the full impact of overnutrition all the cells in the body must be subjected to it to some extent Why should this be so lethal? At one time we were struck by the fact that in *Tokophrya* as in man this decreased the ratio of the nucleus to the mass of the cell and that for some reason connected with this the more vulnerable cells then succumb The

development may be limited and fast growth essential for survival Some of the migrant birds illustrate this very clearly but a little pig like ours would almost certainly fall by the wayside in its natural surroundings long before it had reached maturity even if it has three or even ten times the normal expectation of a pig's life in our little sty Methuselah you will recollect, died at the age of nine hundred and sixty nine years We do not know the age at which he married but he appears to have been one hundred and eighty seven when his first child was born Unless the writer of the book of Genesis forgot to put in the decimal points one may surmise that Methuselah must have had a very sheltered upbringing and not quite enough to eat for his first hundred years or so Unlimited food of the right kind leads to rapid development as you can see from the growth curve of our big pig who was only just over seven months old, yet she weighed 350 lbs and had ovulated three times by this age Rapid growth and development must be an advantage in the world at large but once the vulnerable early stages of an animal's life have passed too much food of any kind is probably a serious handicap to survival in natural surroundings and we know that it is a modest one for a man even in the cloistered world of modern civilisation We clearly want to provide for rapid development in early life followed by a prolonged period of productive adult life, yet the duration of life as a whole appears to be closely related to the rate at which an animal approaches its mature weight (Brody 1945) How to break this natural law appears to be one of the little problems waiting to be solved

These ideas have a bearing on pathology much wider than might at first be supposed If the principle be accepted that the capacity of a cell to reproduce itself is reduced and that its demise is hastened by the accumulation of food materials or calorific reserves within it certain diseases become more understandable Take kwashiorkor for example This is now thought to be a state of extreme undernutrition in a weanling child due to a deficiency of protein, an essential requirement

but natural protection, environment and heredity must all enter into the picture. The type of overnutrition must also matter, and if there is any truth in our speculations about kwashiorkor and alcohol it may be possible to have the cells of one organ dying of overnutrition while the animal as a whole is suffering from undernutrition. It is admitted frankly that these ideas are speculations rather than theories and that even if they are true they are generalizations of the broadest type and there is clearly an immense amount of detailed information to be obtained and integrated with them. This will take time, and we may not live long enough to see it accomplished. If, however, we have provided you with twenty minutes' entertainment and given you something to think about, this is all we can honestly say that we set out to do.

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matter, however, is not quite so simple as that, for the evidence is that our little pig, who was certainly undernourished, had larger liver cells than those of her big sister or of an adult pig. These contained more protein and ribonucleic acid and presumably therefore more cytoplasm. The percentage of fat was lower than it was in the cells of the well nourished pig. We have therefore been led to suppose that the lethal factor is not so much the size of the cell as the nature of the material enlarging it. Too much reserve material inside the cell is the trouble, or perhaps too much inside it for too long. This in *itself* is no "explanation" but it is possible to get a *little* further by venturing into the modern world of cellular organisation and enzyme chemistry. If the metabolism of the cell is directed more to the handling of reserve materials than to the synthesis of protein and nuclear substances, adaptation and increase of all the enzymes required for the former may be expected to take place (Liener and Schultze, 1950, Davies and Yudkin, 1952), as happens if a bacterial colony is subjected to a change of substrate or to some growth inhibitor. This reorganisation must affect both the deoxy- and ribonucleic acids in the nucleus and cytoplasm respectively (Brachet, 1954, Gale and Folkes, 1954) and may displace or reduce some of those enzymes within or without the nucleus responsible for processes essential for growth and reproduction. When now the stimulus for reproduction comes the organisation of the cell is not ready for it and readaptation may take some time. This would lead to a slow initial reproduction rate (as of the large coloured cysts of *Hæmatococcus pluvialis*) and would also explain the possibility of a return to full reproductive activity. The higher organisms are naturally more complicated and all the cells do not succumb equally readily to overnutrition. Some may be protected in various ways. Overnutrition in the higher animals may cause *death from* its mechanical effects and also from humoral causes (Editorial, 1954) but there must be some reason at cell level why overnutrition kills. One would expect highly specialized cells like those of the pancreatic islets to go first.

fruit flies and cantaloupe seedlings Dublin (1953) using the figures of the Metropolitan Life Insurance Company of New York has further indicated that fat people who lose weight live longer We know very little about the effects of overnutrition in childhood but there are strong indications that it may be important For some time past it has been tacitly assumed that the maximum growth of children is the optimum if one child has a greater size than another of the same chronological age and sex there is a tendency to state it is superior Widdowson (1947) in her admirable study of children's diets writes of the weights of girls being in favour of the professional classes with reference to her finding that these girls were heavier than those of the same age in two other classes Morant (1950) writes of the height standards for British children of particular ages improving from decade to decade if not from year to year Are children who are heavier necessarily more favourably placed than children other

an increase in height

children evacuated to England from liberated parts of the Netherlands because they were malnourished children during the famine in Western Holland and malnourished children in the three Western Zones of post war Germany In general these children were nearly normal in height but low in weight In order to compare their heights and weights with standards we searched the literature for these and made the not very original discovery that such standards were of poor quality, for adults they were in general worse But this study of published figures indicated (Sinclair 1948) that insufficient thought has been given to the most desirable rate of growth which is not necessarily the maximum rate We can make a boy of twelve years taller and heavier than he would otherwise be by injecting anterior pituitary lobe extract alternatively we can make him heavier and probably taller by superalimentation There is indeed a tendency amongst nutritionists to regard the child of perfect nutriture as placid rotund red

# TOO RAPID MATURATION IN CHILDREN AS A CAUSE OF AGEING

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My great grandfather, Sir John Sinclair, who was founder and first President of the Board of Agriculture and who for better or for worse introduced the word "statistics" into our language, had a passion for codifying knowledge. He regretted, even in 1807, the large number of printed books and papers, although it is only fair to add that he was himself responsible for 122 volumes: "Such immense masses of printed paper can answer no good purpose, and are a heavy load upon literature and the acquisition of useful knowledge" (Sinclair, 1807). In his four-volume "Code of Health and Longevity" (1807) he gathered together a mass of information relating to the attainment of old age, and in this diet played a conspicuous part. Dr Clive McCay has done likewise in a number of important publications, but unlike my great grandfather he has supplemented his researches with experiments upon lower animals from cockroaches to dogs. Of course others had earlier concluded that a sparse diet prolonged life. The Venetian nobleman Luigi Cornaro (1558) and the Miller of Essex were driven by bad health to adopt a simple diet, but that great pioneer of experimental human nutrition, William Stark, concluded (1788) that they "were driven to temperance as their last resource, and the probability is that nothing but the dread of former sufferings could have given them resolution to persevere in so strict a course of abstinence".

We are all thoroughly familiar with the evidence that over-nutrition and obesity tend to shorten life: this has been demonstrated for such different organisms as man, rats, trout

and increased the duration of life from nineteen days up to twenty nine days Kopec (1928) reached similar conclusions with *Drosophila* caterpillars and tadpoles, and others using protozoa (Rudzinska 1952) *Cladocera* (Ingle, 1933), mice (Tannenbaum 1947) and rats (Riesen, Herbst, Walliker and Elvehjem 1947, Templeton and Ershoff 1949, Sherman, Campbell and Ragan 1949) Carlson and Hoelzel (1946) found that alternate fasting and feeding increased the life span of rats without influencing the growth rate The most extensive and important experiments are of course those of McCay upon trout and rats (McCay Dilley and Crowell 1929, McCay Sperling and Barnes, 1943 McCay 1952) There can be no doubt that in general underfeeding of lower animals during the growing period delays maturation and increases the life span

*If this* is true of most lower animals it may be true of man in whom indeed there is evidence that overfeeding hastens puberty (Bruch 1941 Le Marquand 1951) Further as Brody (1945) showed man differs from other animals in spending a relatively long time in reaching maturity which in this context means ability to reproduce In shortening this period by overfeeding we may well be harming children and shortening their life span McCance (1953) from Cambridge England has supported this possible damage to children by overfeeding we may be shortening the lives of the generation now growing up in this country by trying to make them grow faster with school meals and school milk I concluded much the same in a Cutter Lecture I gave in Cambridge Mass a couple of years earlier (Sinclair 1951) I think we should bear in mind that *the optimum* rate of growth of children is not necessarily the maximum and that harm may be done by excessive feeding of children with milk or school meals and now by medication with vitamin $B_{12}$ and aureomycin even though these activities make them grow more quickly and mature earlier Of course *it* may be maintained that the children are deficient in vitamin $B_{12}$ if they grow faster when this vitamin is administered although presumably the same

faced, and seated in contented contemplation of its folds of flesh During the past several years there has been a marked increase in the rate of growth of children, although in England and the U S the adult male height has remained unchanged It has not been shown that this increase in rate is necessarily advantageous indeed it may be undesirable since the long time taken to reach maturity is characteristic of the human genus' Morant (1950) has assembled an impressive number of data from which he has concluded that the maximum mean height of British men is attained earlier but is not greater than it was a century ago

These conclusions of Morant have been criticised by Boyne and Leitch (1954) The layman of course feels convinced that Englishmen are now taller than their ancestors, they hit their heads on beams of old houses and surviving suits of armour are too small for the average man But the argument, weak as it is from the size of armour might be fallacious because of the tendency for small suits to survive First if the smaller suits were in fact inconveniently small they would be used less and would wear out later Secondly, as ancestors moved from castle to manor to house to cottage, the smallest suits would tend to remain in the family since they would need less cleaning occupy less room and be less valuable as scrap metal

Over a century ago Edmonds (1832) produced 'a new theory of the cause producing health and longevity' He believed that hardship in youth tended to decrease the rate of maturing and estimated that an increase of a year in the duration of infancy could increase the life span by seven years In lower animals various workers have shown that underfeeding during the growing period delays maturity and lengthens life Kellogg and Bell (1903) prolonged by this means the time required for metamorphosis by silkworm larvae, and Pictet (1905a, b) did the same in butterflies Chapman (1920) prolonged the life cycle of *Tribolium confusum* by inanition Northrup (1917) found that inadequate feeding of *Drosophila* during the larval period prolonged this period

subject for research, but it is possible that there was deficiency of riboflavin in some of his diets. Jolliffe and colleagues (Jolliffe, Funaro, Frontali, Maggioni, Corbo and Lanciano, 1953) have been studying the effect of vitamin $B_{12}$ (20 $\mu g$ daily) on the growth of Italian children, and in the initial seven months found a significant increase in weight. In the second year of study (Jolliffe, 1954, personal communication) significant increases in weight have again been found, but no significant increase in height, which is surprising since the INCAP workers concluded (1953) that 'Throughout these studies rate of gain in height has proved the more convincing variable'. In India, it has been found that vitamin $B_{12}$ appears to have no effect upon growth in undernourished children whose diets are inadequate (Annual Report, Nutrition Research Laboratories, Coonoor, 1953).

We do not know why overfeeding produces early maturation. Overweight mothers tend to have large babies, so do diabetic mothers, perhaps because they have a greater ratio of glucagon to insulin and the former stimulates growth (Elrick, 1953). But the longer life span of animals that are underfed during the growing period appears to be a general event occurring in animals that have no pancreas. If length of life span is taken as a criterion, such animals should not perhaps be regarded as 'underfed'; their diet should be regarded as the normal one. Whatever the mechanism, I wish to repeat what I have been maintaining for the past few years that until we know more about the biological effects of overnutrition during childhood we should be careful of concluding that maximum growth is the optimum.

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claim would not be made for aureomycin Indeed we know that it is possible for deficiency of vitamin $B_{12}$ to arise in strict vegetarians The problem is not merely of academic interest since very large areas of the world cannot be and probably never will be supplied with sufficient animal protein to meet optimum demands and the question of supplementing vegetable protein with amino acids that are lacking or with vitamin $B_{12}$ is an important one Several trials have already been carried out in some of which animal protein was compared with vegetable protein, in others supplements of vitamin $B_{12}$ were added to vegetable protein None of these trials is conclusive or satisfactory usually because the test has been of too short duration In short trials Widdowson (1948) and also Gomez Galván Bienvenu and Cravioto Muñoz (1952) found no superiority of animal over vegetable protein For the past three years the Institute of Nutrition of Central America and Panama has been studying the problem (Scrimshaw and Guzman 1953 INCAP Annual Report 1953) no effect of a supplement of animal protein was found amongst children of El Salvador apparently because their diet was deficient also in other factors but in Guatemala the addition of vitamin $B_{12}$ (20 μg) to children on a basic diet of 6 g of animal protein daily produced over a period of eighteen months a highly significant increase in rate of gain in height and weight Others had previously claimed positive effects with vitamin $B_{12}$ (Wetzel Fargo Smith and Helikson 1949, Wetzel Hopwood Kuechle and Grueninger 1952 O Neil and Lombardo 1951 Chow 1951 Wilde 1952 Spies Dreizen Currie and Buehl 1952) although negative reports have also appeared (Downing 1950 Rascoff Danewitz and Norton, 1951 Benjamin and Pirre 1952) none of these tests was conclusive Dean (1953) made a detailed study on undernourished German children of the feeding of plant proteins and considered that adequate substitutes for milk could probably be provided from plant sources noting the beneficial effect of adding a small amount of milk, he suggested that the addition of vitamin $B_{12}$ would be a highly important

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## DISCUSSION

*Olbrich* Dr Sinclair, you compared Dutch English and German children, what was the standard? Did you compare the average height of the population in these different countries?

*Sinclair* That is an extremely important question which concerned us very much when we were studying the heights and weights, because of the enormous differences in different racial groups The Bavarian children are shorter and stockier than the northern children but the standards employed in Germany tended to come from the south from von Pirquet s clinic and he was largely studying Bavarian children who were not typical of the children we saw up in the north Therefore I did select for the chart I showed children whom we regarded as being racially comparable with English children from which our standard was adopted

*Medawar* I should like to ask Dr Sinclair a question about delayed maturation in relation to the increased expectation of life He quoted some figures suggesting very tentatively that one year s delayed maturation might be worth seven extra years of life and he also mentioned Morant s investigation of whether or not Englishmen had grown larger in the course of the last century As I remember it Morant s data showed that Englishmen had *not* increased in size over the past hundred years but that they had reached their maximum size if you take that as an index of maturity no less than five years earlier In other words we are reaching maturity today five years earlier than was the case in about 1850 Should that not be correlated with a rather dramatic change in the mean expectation of life Actually the mean expectation of life at say forty fifty or sixty hasn t changed very much over the last hundred years but in so far as it has changed it has increased There is some inconsistency there which perhaps ought to be clarified

*Sinclair* Yes, I think that is important If we take the reaching of adult size as our index of maturation Morant showed that what appears

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to be happening is that the adult height is unchanged, but that it is reached at a considerably earlier age. Now there are so many factors in preventive medicine that have come in during the last century and have tended to increase the span of life, that it is impossible in the study of a whole population to sort out the possible decrease of the span of life that might be produced by this earlier maturation from the enormous increase that advances in medicine have brought about. One is arguing from lower animals to man in suggesting that in man also earlier maturation, or earlier reaching of adult size, might decrease the span of life, other environmental factors being the same.

*Parkes*: Is it really fair to say that the span of life has increased? As I understand it, the expectation of life at birth has increased greatly, but the expectation of life at seventy is now only about six months longer than it was a hundred years ago, and it seems to me therefore that the expectation of life in the biblical sense at any rate is just about the same, although there are many more people living long enough to expect it.

*Franklin*: Would Dr. Sinclair define maturation?

*Sinclair*: I think that is a question for a biologist.

*Bartlett*: Isn't it the case that maturation cannot possibly be defined in terms of a point reached at the end of a process of change? If the rate of maturation has any relation to length of life, it surely must have to do with the rate of some of the processes in reaching this final point, because one cannot assume that every process that is involved is increasing at the same rate. One would assume also that the earlier age ranges must vary among themselves a great deal. Maturation is not one single process, whatever it is, and I shouldn't have thought it could possibly be defined in terms of a terminal point of some sort.

*Brull*: I feel it will be impossible to answer the question on human beings since they live so long, and there is the progress of medicine and of hygiene. Before you can have the result of your experiment on the children you are considering, medicine will have made more progress. The reason why people live longer is only due to victory over infectious diseases, not to improvement in nutrition. Grown up people eat like fools, or as their government lets them eat, or as advertisements tempt them, but animals are fed scientifically, so studies must be made by comparative physiology; there is no way out.

*Fletcher*: I agree with Sir Frederic, but I should like to ask Dr. Sinclair if he thinks that early maturation in size means also early maturation in capacity for reproduction, for instance.

*Sinclair*: I had that in mind, naturally, but I think size is an easier criterion for measurement, though of course it is only a small part of maturation. We also took another criterion, namely the onset of menstruation, and tried to get information about that in populations that are undoubtedly on different planes of nutrition, but we have run into the difficult problem of primitive undernourished girls not knowing their ages. It is very difficult, as you are only too well aware, to find out what has happened in past years because the figures are not obtainable. I had hoped that I could get some of these figures from my own college

in Oxford, because for centuries past we have had boys singing in the choir, and I have unsuccessfully searched everywhere in our very good records to find out the ages at which choristers ceased to be choristers when their voices broke

*Krohn* But the reverse is also true, that if you feed animals too well they are poor breeders and begin to breed later than normal

*McCance* Yes, that is true We are all interested in our department in the fact that show animals may not breed at all That is one reason, I believe, why show breeders like to sell their animals at the show, for

he has written a paper precisely on that point

*Parkes* You mean they lived longer than they would have done if

... of life, there is a moralistic factor here that it *shouldn't* be healthy to eat well, or to smoke, or to indulge in physically pleasant activities We never hear of the fact, which seems to be true, that alcohol in moderation is conducive to longevity

*Parkes* I can su...
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... Dr Sinclair whether the restricted diet during and ... effect in this country? I ...

restricted in Britain for millions ... see any results. Have there been ...

... that chronic underfeeding ... We have ... st to death. ... ong time I ... ntly follow- ... dicap in the war

Nicolausen In the last year or two considerable ... ion has been ... and Norwegians kept in German concen- ... effects are ...

... of these people ... now trying to do it systematically in Nor... surprised to see what they could tolerate

Cordry It will take time, of course ... Dutch people who were starved during the ... quite a ...

Sinclair That ... ficult to analyse because in the famine in the Netherlands ... so many other relevant factors, such as who was admitted to hospital But I think it is generally agreed that the incidence of eclampsia was considerably decreased

... discussion about whether the slow ...

... years

... McCay talked of many animals but didn t mention the one that I m particularly fond of, and that is the deer The deer doesn t live very long only fifteen years, and has one important characteristic it

to 18 or 19 g and were large round spheres, so heavy that they couldn't walk. However, so far as I remember, the age at maturity didn't appear to have been altered

*Verzar* I think we have again reached a very basic question of old ... want to get on ... *ing, statistical* ... t would only be ... can't compare ... ld propose an organization which could keep ... on ageing on a definite group of individuals

*C* ... we can't ... ugh ... arly

... ous one is ... ch are the ... situation ... it may be analogous ... that which ... population of rats and divide them ... inst each other

... tuberculosis is checked ... will tend to grow very slowly and ... bit of confusion with regard to the effects of retarded growth amongst a group of rats of that type, where one has diseased and healthy animals

... It is particularly brought home to me by seeing some ...

case of the vigour, ... development goes with longevity and with long reproductive life. You get the sort of mouse (I think it was one of Gates's crosses) that breeds earlier than anybody else's mouse and also seems to live *longer and goes* on breeding longer than anybody else's mouse. Unless we know what the gene ... population is we may be misled by that sort of ... and an ... heterozygosity.

evidence for an age change in basal metabolism disappears.* So I am inclined to question whether there is an actual slowing of the cellular ... oxygen consumption ...

metabolising tissues.

*Cowdry* However, it used to be thought that there was a significant dehydration reduction in amount of water per unit of weight in older tissues. And then Hastings and others came along and seemed to show that this was not the case. What is the situation now?

*Shock* I think there is no question but that the total water content of the animal decreases with age. Using anti-pyrene as an index we have found an average change from 34 6 l. to 29 7 l. between the ages of forty and ninety years. Again, if you want to indulge in some fancy ... of function or protoplasm ...

age

*McCance* I don't know anything about the water but if you starve a person over eighty he derives exactly the same percentage of his basal calorie requirements from breaking down of his own tissues as a young adult does. So right on into old age that balance seems to be preserved.

*Cowdry* Can you say that the basal metabolic rate in females is in general somewhat lower than males for the same age and that that is in some way related to increased life span?

*McCance* Our work has all been done on old men, and I'm afraid I can't tell you anything about old women.

*McCay* There is no *proof* of any sex difference in any animal other than man.

*Cowdry* There is a difference in man, isn't there?

*McCay* Apparently yes.

... the female has more fat on her from the start, so that this ... amounts of fat. Unfortunately we ... if one were ... would be any sex difference ...

*Sinclair* I think Ancel Keys has come ... s no sex difference in terms of active metabolising tissue—it is that there is more fat.

*Cowdry* But there would be a difference in terms of total weight?

... Or surface area

... International Conference on ... of ... this

* Shock, N. W., Yiengst, M. J. and Watk... 338. See also Shock, N. W. in Symposium on Problems of ... logy, National Vitamin Foundation, Nutrition Symposium Ser. No. 9, 1954

regenerates its antlers every year. Antlers can be collected and weighed and analysed or even studied when they are growing. It is a beautiful animal for studying the ability to regenerate. Here is a reproducible growth impetus every year; it goes down gradually as the animal grows older. But you can change the development of the antlers; you can control it by changing the endocrine environment.

*Lansing:* There is a very large literature on the rôle of nutrition in experimental animals. It doesn't need another fifteen years to be resolved; it has been resolved for more than fifteen years—some of the work was done in about 1900—on silk worm, *Cladocera (Daphnia* in particular), on rotifers, on *Planaria* and other flat worms, even on rats and mice. Hoelzel and Carlson several years ago published extensively on this subject, and I'm surprised at the amount of general agreement there is. The consensus is, with amazingly little difference of opinion, that restriction of the diet prior to maturation does seem to effect a prolongation of life span; restriction of diet, or super-alimentation after maturation having no effect at all.

*Shock:* But isn't the problem that we are raising here a little deeper than that? We are asking: what is the mechanism whereby this process is carried out? It seems to me that although we do have a fairly large body of information on various animal species, we have not yet any fruitful ideas on the mechanism of the process.

*McCay:* In the other direction (and in regard to Dr Olbrich's question) we have left a substantial group of rats to become middle aged and fat and then forced some of them to become thin by exercise or restriction of food, and that lengthens the life of the rat; not as significantly as retarded growth, but one does get a significant result.

*Tunbridge:* You have some work on your mental patients, haven't you, relating to the calorie requirements in elderly people? Is it correct that their caloric intake is diminished?

*McCay:* There is work on that, but I didn't do it. That gets involved with basal metabolism. It looks as if evidence is very good that the BMR drops in older people, but I've never found any sound evidence for any experimental animal that the BMR drops with age. Assuming those data are sound, man may be unique in that respect.

*Shock:* I think Davis (*Amer. J. Phys* [illegible] 19[illegible], 119, 28) studied the basal oxygen uptake of [illegible] found a reduction in the basal [illegible] you'll find one can jump either way.

*Shock:* We have collected evidence on changes in basal metabolism with age, and it is true that the oxygen uptake of the total animal diminishes as the individual gets older (Shock and Yiengst, 19[illegible], *J. Geront.*, in press). But if one uses some other [illegible] of functional tissue present [illegible] standard [illegible] We hav[illegible] individu[illegible] —and [illegible]

e lance for an age change in basal metabolism disappears* So I am
, · other there is an actual slowing of the cellular
, · a drop in oxygen consumption
, or cells and
slowly
metabolising tissues

*Condry* However, it used to be though . gnificant dehydration, reduction in amount of water per unit of weig in older tissues And then Hastings and others came along and seemed to show that this was not the case What is the situation now?

*Shock* I think there is no question but that the total water content of the animal decreases with age Using anti pyrene as an index we have f change from 34 6 l to 29 7 l between the ages of · on want to indulge in some fancy · protoplasm, at least in our group ion of the histologically observed loss of sing age

*McCance* I don t know anything about the water but if you starve a person over eighty he derives exactly the same percentage of his basal calorie requirements from breaking down of his own tissues as a young adult does So right on into old age that balance seems to be preserved

*Condry* Can you say that the basal metabolic rate in females is in general somewhat lower than males for the same age and that that is in some way related to increased life span?

*McCance* Our work has all been done on old men, and I m afraid I can t tell you anything about old women

*McCay* There is no proof of any sex difference in any animal other than man

*Condry* There is a difference in man isn t there?

*McCay* Apparently yes

· ale has more fat on her from the start, so that this mounts of fat. Unfortunately we · now if one were would be any sex difference

*Sinclair* I think Ancel Keys has is no sex difference in terms of active metabolising tissue—it is that there is more fat

*Condry* But there would be a difference in terms of total weight?

*Sinclair* Yes of course Or surface area

*Brull* May I remind you that at the first International Conference on Gerontology I reported that we had started a study of the influence of nutrition and feeding on the ageing of inbred mice We started this

* Shock N W Yiengst M J and Watkin D M (1953) *J Geront*, 8, 338 See also Shock N W in Symposium on Problems of Gerontology, National Vitamin Foundation, Nutrition Symposium Ser, No 9, 1954

seven years ago, and it is still in progress. Of course we lived with the illusion that we can avoid disease. This illusion covers all studies in gerontology, and will remain an illusion because we shall never be able to study pure ageing without the influence of exogenous factors.

To go back to Dr Lansing's contribution about changes in the constitution of " "
whole animal
diet and then
these analyses is that the main effect of ageing is the putting on of fat and lipid substances. But this is already known. One of my previous masters, Terroine in Strasbourg, demonstrated thirty years ago that when you analyse tissues of animals, if you are to have comparative figures you must starve your animals to death before you analyse. When you do that, and you take kidneys or liver or muscle of different animals of the same species, the results are comparable. So we are going to do that. But as regards comparing results or exchanging of possibilities, we could just as well exchange powdered animals. I shall do what I can to analyse the powdered animals from many points of view, I shall do gross analyses and some of the amino acids etc., but many other laboratories may do what I am unable to do. So if we are thinking of working along those lines we might help each other. I have not published anything yet about these mice, perhaps I never shall, but I am ready to compare and to exchange powdered mice of that inbred colony with anyone who wants help in that way.

# PSYCHOLOGICAL ASPECTS OF AGEING

SIR FREDERIC BARTLETT, C B E , F R S
*Applied Psychology Research Unit Cambridge*

ABOUT seven years ago the Nuffield Trustees, with the approval of the University, established a group at Cambridge for the experimental psychological study of the effects of ageing in the case of normally fit persons It is a few of the chief results achieved by this group which I am now going to describe and consider The principal merit for the achievements is due to Mr A T Welford, the Director of the Group, and to his fellow workers If there are mistakes of interpretation they are mine

It was from the beginning agreed that we should not be much concerned with the extremely old but rather with the critical age ranges from round about forty five to sixty five It was also agreed that we should not confine our attention to relatively isolated items of behaviour such as sensory threshold measures reaction times, immediate or remote memory span disconnected from any other activity We should rather look at these in that intimate association with continued work which they have in practically all forms of day by day actual behaviour This meant that our main pre-occupation must be to try to find out something about the nature and conditions of skilled behaviour, whether of body or mind and that our initial problems would concern demonstrable changes in skilled activities which normally occur with advancing age

We had of course some guide both from the results of a great amount of earlier experimentation and from commonly held opinions Nearly everybody for instance agrees that most actions are slowed up with increasing age and many experimenters had already reported this But when does this

slowing up begin? Once begun does it continue and perhaps increase without recovery? Particularly are there certain phases of complex skilled activity which more than the others exhibit this slowing up?

It appears that there is some slowing up of skilled actions normally in the late twenties This is followed by considerable or complete recovery, but it reappears in the late thirties or middle forties There is again recovery until the early or middle fifties, and then the slowing up seems generally to continue and perhaps to increase But there is nothing very remarkable or dramatic about it in the case of the fit person and if it is adequately countered probably it need have no important practical significance I am speaking of course of skilled performance which must be done rapidly, but does not require any great expenditure of muscular effort If the latter is required, and still more if work has to be done at relatively rapid rates and with accompanying overall bodily mobility the final slowing up appears earlier, usually in the early forties and with little or no subsequent recovery

Very much more interesting is the question of whether there are certain stages in the performance of skilled movements of all kinds which are especially susceptible to change It rapidly became clear that overall time measures of performance may be extremely misleading (Welford 1951) For example in one of the early experiments a small object was set into apparently random movement over a surface marked off in small squares When it came to rest its position had to be accurately identified by manipulation and if this was done successfully the object was set into further random movement and so the experiment proceeded If the overall time was recorded for a series of correct locations it could, as indeed anyone would expect be exactly the same with great and significant differences in the internal pattern of manipulative movement It was from this experiment that we got the first suggestion that the most unstable element in any series of accurate adjustments to changing stimulation is not the speed of movement, and not the reaction time (if

this is defined in the usual way as the interval which elapses between the appearance of a stimulus or signal for movement and the beginning of the movement) but the resting time, or recovery time, between an adjustment which is in one direction and an immediately following adjustment in a different direction (Leonard, 1953) This has since been confirmed in many other experiments, and, recently, in an analysis of time motion study films It is the stationary items in apparently continuous movement series that are most liable to change They are the constituents that are most readily accelerated or decelerated They tend to be drawn out under the influence of certain drugs of fatigue, and, almost certainly, of increasing age

The interesting thing is that these stationary constituents in a flow of movements, or of ideas, occur almost always, perhaps always, where something specific about signals for action has to be perceived such as the shape, size, colour, disposition and number of objects, or where, in what is usually called mental" skill, there is a shift from one topic to another, or some new consideration has to be brought into a process of thinking This is to say that the most variable elements in skill, and the ones that are most likely to be affected adversely with increasing age, unless precautions are taken are just those that most indubitably demand central, neurological activity and control

To have demonstrated an increasing importance of halts in a flow of movements or ideas, with increasing age is a step in advance But by itself it is not much of a step, and when we try to go further we are at once brought up against difficulties of interpretation which can be resolved only by more experimentation

There is considerable evidence now that older people—I mean broadly from the middle forties upwards—tend to look both at what they are doing and at the signals for what they are to do in most kinds of bodily skill, when accurate and rapid work is required far more than younger people (Szafran, 1951) It may even be the case in general that increasing

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of skilled response within fairly wide age ranges One characteristic of behaviour in the middle age ranges and upwards is an unusual persistence of mistakes If a mistake has been made once or twice there seems to be a strong tendency for the performer to move to the required response by way of the unwanted one, even when it is perfectly clear that the latter is unwanted (Kay 1951) This is just what would be expected if the course of an action is determined at a relatively early stage of the action

Supposing that there were both some retraction of anticipation range and some advance of the point of no return all the slowing up of skilled work which has been experimentally demonstrated would be fully accounted for Whether they do occur and if so by what human mechanisms they are effected are questions perfectly amenable to an experimental approach but the experiments have not yet been done

One final question about these results of which I have spoken and a good many others both in practice and in training which I might have discussed had there been time are they inevitable? That depends upon the meaning which is attached to inevitability If inevitable means results that will occur unless special precautions are taken they are If inevitable means results that are bound to occur whatever is done about them simply because everybody advances in age the strong probability is that they are not But that opens up another and perhaps a still wider, field for experimental study

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## DISCUSSION

*Shock* In investigating problems of this kind can one deal with a wide variety of skills or is it necessary to confine the tests to fairly definite acts? In other words how broad an experience can one deal with experimentally in this kind of approach?

age prefers to have cues for performance and of the course of performance from as many sources as possible. In particular it may be that sensitivity for proprioceptive feed-back diminishes. For this however there is no direct evidence as yet, or, in fact, of significantly lowered threshold response in any direction. It is safer, though of course less satisfying, to treat the demand for a greater amount and diversity of confirming evidence as one of the signs of the increased caution which is alleged to accompany advances in age.

It is more intriguing to consider the possibility that the range and function of anticipation may change with age. We have decisive experimental evidence that far and away the most important character in skill performance is what has to be called its temporal structure. The way in which any item of skill is dealt with depends upon its relation to preceding and succeeding items in the sequence of which it forms a part. If a signal for action is given at an appropriate interval before the moment of action it is the resting time between movements in one direction and another, whether of muscle or of mind, that is significantly reduced. It is a reasonable suggestion that the general effect of accumulation of experience is to enhance the influence of preceding items in any skilled series and to depress that of succeeding items. There may therefore be a retraction of anticipation range with age and if this is so it would undoubtedly produce just that preference for shorter advances and more and longer halts which older performers are known to show. But only further experiments can decide.

There is yet another possibility. Every human action exhibits a character which may be most forcibly described as a 'point of no return'. That is, it reaches a phase beyond which putting in new signals produces no effect whatsoever. Before this stage is reached there is a range within which incoming signals produce actions, but the action is maladjusted, a mistake is made. It has become a matter of great theoretic and practical importance to determine both the absolute and the relative 'points of no return' for all kinds

*Bartlett* It is quite true that from the fifties upwards, the attempt to introduce new conditions of performance, new types of stimulation is likely to be met by a relatively long learning stage But I think that is about the only thing that we can say about that Most of the work that we have been doing involves repetitive activities which have been completely learnt

*J Verzár* In that connection, and relative to Dr Welford's work, we have been doing some maze experiments with rats We have tried to study two problems: learning at different ages in a multiple T maze, and relearning or memory of it at different stages during life We have found slightly lower learning in extreme old age, but the most interesting thing was that certain of the old animals, about 40 per cent of the groups, were not able to learn at all when presented with the maze for the first time in old age, whereas the other 60 per cent learnt it within the normal range although rather more slowly than the young rats The second point was that in memory tests with groups of rats which had learnt the maze at different ages, again in extreme old age about 40 per cent forgot the task which they had *learnt* in earlier life, and were quite unable to remember it whereas the other 60 per cent of that same group remembered as well as the *younger* rats I don't know how far that correlates with the work of Dr Welford

*Bartlett* I didn't say anything about the learning side of all this, [illegible] take special precautions what you discovered [illegible] beings That is to say the [illegible] older age are [illegible] relatively young If the older [illegible] nditions of learning as the young they either learn [illegible] haps fail to learn at all But so far the evidence is that this is more a question of the conditions under which the tests are given than it is of any absolute capacity to learn It seems extremely likely that as far as human beings are concerned, there is no particular reason why people shouldn't go on learning *things* as long as they live, or at any rate *up to* extreme old age provided they are given conditions of learning which are suitable for their particular range

*I think* too there is a great deal of agreement between the results about relearning which you have indicated with the rats and similar cases with human beings The *only* thing about it is that it seems as if we can get rid of all these differences if you adjust *your* learning conditions to the age range that *is* concerned It is very difficult to do that with rat experiments I think, but a little *easier* to do it with human experiments The whole programme of our unit was to be concerned also with learning and we have got a good deal of evidence about the learning processes which are characteristic of age ranges, and they certainly are different in human beings for different age ranges, but I haven't *time* to say anything about that.

*Parkes* I suppose in the case of the animal experiments the stimulus applied to learning is the same for young as for old, is it? *Loss of food*, or falling into a tank of water, or whatever?

*Bartlett* For the most part, our initial work was carried out with skills that involved the notion of tracking You have an object which is moving in a given direction, and its movement either has to be progressively followed or controlled in some way. Then there was an extension from this, and the things that I have spoken of have been tried out with a considerable variety of bodily skills, with a good many mental skills involving memory and anticipation processes proper, and they have also been taken into the factory and applied to repetitive activities such as are involved in assembly work and conveyor belt work The same features have occurred over and over again The thing which is variable is the interval between a movement that is required to locate something in one position, let us say, and then take it from that position to another The same thing occurs in connection with a lot of skills in games, which we have studied It also occurs in a good many operative procedures in the laboratory, where what you have got to do is to locate a particular thing that you want to do something about and then move it into a given position in relation to something else A skilled process always involves a sequence of movements mental or physical interspersed with changes of direction, and it is the halts between changes of direction that are the most unstable feature, it is these which are most affected when what we call tiredness or fatigue sets in, which are most influenced by drugs, and which become more marked with increasing age

*Franklin* Is there any difference between the sexes?

*Bartlett* None at all, as far as we know, but most of these experiments have been done with men in the factory and in the laboratory But wherever we have had older women they have shown the same characteristics, and there doesn't seem to be any important difference at all

*Lewis* Have any of these studies been carried out on people whose mental powers were obviously failing—perhaps in the early stage of dementia?

*Bartlett* No we try to keep away from that as far as possible and to have a range of people who would satisfy the requirements of normal mentality We have kept off people with special disabilities because we think that complicates the picture at this stage I think it possible that you would have these things happening but a good many other things superimposed on them as well

*Lewis* I was wondering whether since these are central happenings, they would be more evident in people with dementia

*Bartlett* Yes, it could be but I don t know about that

*Franklin* How far does the ageing of the eye come into this?

*Bartlett* Since we have tried to keep to people with normal eyesight, we have no evidence that anything very much is dependent upon visual responses as such, except that the optimum distance of the signal for action from the viewing point will tend to go a bit further away

*Shock* Do you think that the way to look for age changes is in an activity that is well practised in the individual, one that he has done repeatedly? Or are you more likely to see age differences when you present him with an entirely new activity?

*Parkes* In general are assembly line types of jobs unsuitable for old people? Would it be too awkward to ask whether there is any evidence that the increasing expectation of life at birth is bringing any increased expectation of potential working life?

*Bartlett* I don t think it would be possible to give an absolutely categorical answer to that The indications are that the increasing expectation of life has already lengthened the potential working life, when you mean by the potential working life the period during which an individual may be of economic service in a community I think this is true but it is an opinion rather than anything that can be definitely proved. But it seems to me certain that the two things must go together because the increasing expectation of life is simply an expression of increasing fitness of human beings. At least I think that is true I don t believe—this is again a matter of opinion—that the increasing expectation of life is very much tied up with the fact that the older people are being better looked after I think it is that they are potentially much more active than they used to be

*Parkes* The increasing expectation of life is presumably an expression of the increasing control of diseases which killed children or adults in their prime not of the control of senescence

*Bartlett* The question of whether in fact because people could go on working for longer with satisfaction to themselves and with economic gain to the community they will be expected to do that is quite another matter of course I think they will have to be

*Schulze* I wish to mention a problem of the psychology of ageing, which seems to be remarkable to me that is the changing of the psychical conception of the speed of time as a function of ageing All of us will remember that when children we considered a holiday lasting six weeks an almost infinite space of time As adults we experience now an apparent shortening of our idea of this period In old age we shall feel the same space of time to be a trifle

This fact may correspond to the change of our mental attitude to the course of time in general In childhood we are living merely in the present and actual events seize upon our fancies When we grow adult the future achieves more and more predominance in our minds whereas the elderly are looking back and lose their power of recollection of recent events This quick motion change of our spiritual idea of time flow is a very interesting phenomenon and I wonder if any experimental work has been done in this field to realize the acceleration of our internal chronometer

*Bartlett* I think that there are a lot of investigations about this but in so far as these have been experimental they are not terribly convincing It is very difficult to find any way by which you could get experiments giving clearcut measurable results.

*Shock* This is rather an expression of the Weber Fechner law, isn t it? The boy s total lifespan is ten years so that proportionately a week represents a large part of it whereas the fifty five year old individual has a lot of years to base his judgment on, and a week is only a small portion of his total life experience Accordingly, one would expect the

*F. Verzár*: Yes. It was hunger motivation—not very extreme daily fasting.

*Parkes*: And that again makes the penalty for failing to learn quite different from what might be applied to human experiments.

*F. Verzár*: Yes. But we were interested in these individual differences: that some old beasts could still remember and others had total loss of memory.

*Parkes*: Does that kind of sanction mean the same to an old animal as to a young one?

*F. Verzár*: There is admittedly the possibility of an age difference there.

*Parkes*: If I remember rightly, Sir Frederic, one of the original ideas behind this project was to find out what particular jobs old people might be suitable for. I take it that the answer is still rather in the future, is it?

*Bartlett*: That is quite true, but I think the very general answer is that provided one avoids the difficulties inherent in a great many current industrial processes of rapid forced pacing, there are probably practically no jobs for which the upper age ranges are not suitable.

*Parkes*: Provided they are taught appropriately or allowed to take things at their own pace?

*Bartlett*: Provided conditions of operation are readjusted at the right age range. If one looks at the structure of British industry—if we are talking about the practical side of this—at the moment, one of the most astonishing things is (it is reported over and over again, and is I think quite definitely established) that there is a very great proportion of older people doing heavier work and doing it successfully. I didn't say anything about heavy work, but in point of fact if a man remains fit he seems to be perfectly capable of going on doing heavy work, provided he can do it in his own time. A great amount of the heavy work in industry at the moment is done at the operator's own pace. So that is one of the many reasons which tend to make the heavy work be passed over to the older people.

*Parkes*: Does that mean that old people now are more capable of doing heavy work than old people were previously?

*Bartlett*: No. I don't think there is any reason to think they are more capable of doing heavy work. They are more capable of doing slow work, and most heavy work is slow work. There are other reasons [illegible] is that all the heavy jobs in a modern industr[illegible] unmechanized, and all the f[illegible] people getting [illegible] who haven't [illegible] That is going [illegible] or because there are fewer and few[illegible]. Therefore if one is looking at this thing in a pr[illegible] sense, the really important thing is not to bother very much about the heavier work at the moment but to find out what could be done, given proper conditions, with the type of activity which is obviously going to predominate in the community in a few years' time.

# ADRENOCORTICAL REACTIVITY IN AGED SCHIZOPHRENIC PATIENTS*

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PREVIOUS investigations by Pincus *et al.* (1949) and Hoagland *et al.* (1953) have described abnormal findings in adrenocortical responsivity in young and middle aged schizophrenic patients. The present paper deals with a study of adrenocortical reactivity in elderly normal and schizophrenic men to determine whether senescence modifies the results obtained in the younger groups.

The normal subjects included 34 men, ranging in age from sixty to ninety one years with a mean of seventy six years, who were obtained partly from the community at large and partly from a home for the aged. All were active and were free from any obvious disease except the usual stigmata of the aging process. The patients, 33 in number, were all residents of the Worcester State Hospital. They ranged in age from sixty to eighty one years with a mean of sixty eight years. They had been confined to the hospital an average of 31.3 years, the range being 10.1 to 49.6 years. The majority of these patients were of the paranoid type, with a scattering of the other usual subtypes. All the patients likewise, for their age, were in good physical condition. They were a selected group in so far as co operation was an essential element for the completion of the studies. Data on young subjects are included in this study to exemplify specific points as to the aging process.

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boy to regard a week as much longer than would the fifty five year old man

*Bartlett* I think the truth is that anticipated time and remembered time—if we mean by time a time span as measured by a clock or something of that kind—appears longer to the young and shorter to the old but experienced time that is the span within which we live appears shorter to the young and longer to the old To a young child, what to an old man is a small period of anticipated time seems long, or a small period of remembered time may seem long, but to the old man what is a relatively short period of lived time may seem to be long relative to what it is in the case of the child However, usually this is held to be dependent upon the number of items that fill up an interval You can do this experimentally, if you like, if you have an interval which is started by a sound and terminated by another sound and if you fill this with a series of sounds, then the apparent length of the interval is dependent to a large extent on the number of sounds that are used to fill up the interval If you don t fill it up at all, then it always seems a much longer interval I think that is true for all ages but in general the intervals in a young life are less fully filled up than they are in the older life, until you get to retiring age, when time then may appear to go more slowly

*Lewis* I think it has been found that people s subjective impression of how time is passing is not related in any recognizable way to their actual judgment of how much time has passed between two specified intervals

*Bartlett* Yes, that is the case

age categories. The basal values for the young groups are almost twice as great as the corresponding values for the old groups. This is to be expected on the basis of the greater muscular mass of the young subjects, although a renal factor may be implicated. The normal subjects, both young and

**Table I**

MEAN CREATININE VALUES FOR YOUNG AND OLD NORMAL AND SCHIZOPHRENIC MEN DURING ACTH AND GLUCOSE TOLERANCE TESTS

| ACTH | | | | |
|---|---|---|---|---|
| Age years | Subjects | Urinary Creatinine (g /24 hr.) | | |
| | | Basal | 2 hr | 4 hr |
| 19-39 | Normal | 1.82 [1] | 1.8? [?] | 1.83 [21] |
| | Schiz | 1.54 [28] | 1.57 [27] | 1.42 [8] |
| 60-90 | Normal | 1.08 [18] | 1.40 [19] | 1.20 [19] |
| | Schiz | 0.83 [33] | 1.21 [35] | 1.37 [30] |

| GLUCOSE | | | | |
|---|---|---|---|---|
| Age years | Subjects | Urinary Creatinine (g /24 hr.) | | |
| | | Basal | 1 hr | 3 hr |
| 19-39 | Normal | 1.80 [25] | 1.95 [24] | 1.93 [28] |
| | Schiz | 1.6? [?] | 1.65 [26] | 1.71 [29] |
| 60-90 | Normal | 1.04 [?] | 1.37 [25] | 1.45 [21] |
| | Schiz | 0.91 [34] | 1.41 [34] | 1.44 [43] |

N.B. Raised figures indicate number of subjects.

old excrete slightly more creatinine in the basal state than the corresponding patient groups but the difference is statistically significant only between the two groups of young subjects.

The administration of ACTH results in no essential change in the values for the younger groups but does show a definite increase in the amounts of creatinine excreted in the two

Two test procedures were used: (1) the injection of 25 mg. of ACTH intramuscularly as a direct means of studying the effects of the stimulated adrenal cortex, (2) the ingestion of glucose, using the Exton-Rose technique, which acts on the adrenal cortex presumably via the anterior pituitary. In the ACTH test, under fasting conditions, measurements of lymphocytes and eosinophils were made immediately prior to the injection and one-half, two and four hours after the injection. Urine collections were made from the period of awakening (after discarding the first morning urine) to the time of the injection (average time two hours), for two hours after the injection, and for another two hours. In the case of the glucose tolerance test, the lymphocyte and eosinophil counts were made before the ingestion of the first 50 grams of glucose, a half hour later just before the ingestion of the second 50 grams of glucose, a half hour and two hours after this. Urine collections were made for two hours before the onset of the test and collected during the first hour and the succeeding two hours after the ingestion of the first dose of glucose. In the urine collections analyses were made for creatinine, sodium, potassium, 17-ketosteroids, corticosteroids as neutral reducing lipid and inorganic phosphates. The two tests were done three days apart, the glucose tolerance always being performed first so that no residual effects would be obtained in the second test.

Before discussing results which deal largely with urinary metabolites, a first consideration in the aged should be an evaluation of the efficiency of the renal excretory apparatus. Previous investigations by Shock (1946), Davies and Shock (1950) and Miller, McDonald and Shock (1951) have indicated a decrease in the functional capacity of the kidney in the aged. It is with this point in view that we have analysed some of the data concerning the excretion of creatinine.

The mean values for urinary creatinine during the ACTH and glucose tolerance tests are shown in Table I. The subjects are divided into two groups on the basis of age, and further subdivided into normal and schizophrenic men in each of the

Table II

RELATIONSHIP BETWEEN BASAL VALUES FOR CREATININE AND OTHER URINARY METABOLITES IN ELDERLY MEN

| *Urinary metabolites* | *Corr coefficients** |
|---|---|
| Creatinine vs 17 ketosteroids | +0 68 |
| vs cortin | +0 65 |
| vs uric acid | +0 92 |
| vs sodium | +0 84 |
| vs potassium | +0 67 |
| vs inorgan. phosphates | +0 73 |

*All correlation coefficients are statistically significant

and uric acid This degree of co variance furnishes additional evidence for the renal origin of creatinine variation in the aged As a result of the excellence of the relationships, we have analysed all the urinary data on the basis of creatinine ratios and they will be so presented

The mean values for the two groups of subjects during the ACTH test are shown in Table III

The normal subjects have higher initial levels of eosinophils a finding noted in younger groups (Pincus *et al*, 1949) The difference is not statistically significant, however, in this group The eosinopenia follows similar trends in both groups again with no statistical significance This too, has also been noted in younger groups of subjects by Pincus *et al* (1949) and Hoagland *et al* (1953) The *response* of the eosinophils to the administration of ACTH cannot be used, therefore as a point of differentiation between normal and schizophrenic subjects at any age Ageing itself apparently, has no effect upon the basal eosinophil level according to Olbrich (1947) or upon the eosinopenic response to ACTH (Solomon and Shock 1949) In connection with the magnitude of this response (a 48 *per cent fall*) note should be taken of the diurnal variation in eosinophils observed by Stevenson Metcalf and Hobbs (1953) that from 8 00 a m

older groups An analysis of the change in values from the basal level to the two and four hour readings shows a statistically significant difference between the young and old schizophrenic subjects but not between the young and old normal men

The greater excretion of creatinine following ACTH in the aged may be influenced by the differences in the initial levels between young and old There is a negative relationship between the basal values and the change in four hours so that the lower initial values show greater changes than the higher basal values Correlation coefficients for the four groups are as follows young normal, −0 23, young schizophrenic, −0 CC old normal, −0 67, old schizophrenic, −0 66 Only in the young normal group is this relationship not significant

The data on creatinine excretion following the ingestion of glucose show the same trend as with ACTH (Table I) Again the young subjects, both normal and schizophrenic exhibit no significant change in values over the experimental period while the old subjects show a distinct increase The increase in excretion at the three hour point is significantly higher in both older groups than for the corresponding younger subjects Further the relationships between the basal values and the changes at three hours are of the same order of magnitude as in the case of ACTH

It may be that the initially higher values of the young subjects represent not merely the degradation products of greater muscular activity but also a more efficient renal mechanism which under stress is able to maintain a given level of excretion The impairment of renal function in the old may be manifested in an inability to resorb the increased amount of creatinine presented by the stimulating agent

Not merely does creatinine vary but the other urinary metabolites vary with it In Table II are shown the relationships between basal values for creatinine and the other functions studied, in the old normal and schizophrenic subjects The correlations are of a good high order of magnitude, the highest relationships being obtained with phosphates, sodium

Table II

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| Urinary metabolites | Corr coefficients* |
|---|---|
| Creatinine vs 17 ketosteroids | +0 68 |
| vs cortin | +0 65 |
| vs uric acid | +0 9? |
| vs sodium | +0 84 |
| vs potassium | +0 67 |
| vs inorgan. phosphates | +0 73 |

*All correlation coefficients are statistically significant

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The mean values for the two groups of subjects during the ACTH test are shown in Table III

The normal subjects have higher initial levels of eosinophils, a finding noted in younger groups (Pincus *et al*, 1949) The difference is not statistically significant, however in this group The eosinopenia follows similar trends in both groups again with no statistical significance This, too, has also been noted in younger groups of subjects by Pincus *et al* (1949) and Hoagland *et al* (1953) The response of the eosinophils to the administration of ACTH cannot be used therefore as a point of differentiation between normal and schizophrenic subjects at any age Ageing itself, apparently, has no effect upon the basal eosinophil level according to Ulbrich (1947) or upon the eosinopenic response to ACTH (Solomon and Shock 1949) In connection with the magnitude of this response (a 48 per cent fall) note should be taken of the diurnal variation in eosinophils observed by Stevenson Metcalf and Hobbs (1953) that from 8 00 a m

to noon there is a decrease of 21 per cent in the eosinophil count.

The lymphocyte values (Table III) likewise show no significant differences either in initial or in the post-injection

**Table III**

VALUES FOR BLOOD AND URINARY METABOLITES IN ELDERLY NORMAL AND SCHIZOPHRENIC MEN DURING ACTH TEST

| | Variable | Subject | Basal | ½ hr | 2 hr | 4 hr |
|---|---|---|---|---|---|---|
| Blood | Eosinophils | Norm<br>Schiz | 107[18]<br>126[14] | 180[18]<br>108[14] | 139[18]<br>87[14] | 123[18]<br>61[14] |
| | Lymphocytes | Norm<br>Schiz | 2527[18]<br>2933[14] | 2491[18]<br>2394[14] | 2214[18]<br>2149[14] | 1924[18]<br>2079[14] |
| Urinary Excretion per 24 hours | 17 KS (mg.) | Norm | 5.64[18] | | 3.83[19] | 4.33[18] |
| | Creatinine (g.) | Schiz | 5.17[14] | | 4.67[14] | 4.73[14] |
| | Cortin (mg.) | Norm | *2.34[17] | | 2.14[17] | 2.51[17] |
| | Creatinine (g.) | Schiz | *3.13[13] | | 3.17[14] | 3.13[13] |
| | Uric Acid (mg.) | Norm | 455[17] | | 512[19] | 574[17] |
| | Creatinine (g.) | Schiz | 440[14] | | 490[14] | 519[14] |
| | Sodium (mg.) | Norm | 3324[18] | | **4777[18] | **5409[18] |
| | Creatinine (g.) | Schiz | 3312[13] | | **3524[14] | **2980[13] |
| | Potassium (mg.) | Norm | 2553[19] | | 3301[18] | **4987[17] |
| | Creatinine (g.) | Schiz | 2352[13] | | 2692[14] | **3027[14] |
| | Phosphates (mg.) | Norm | *577[18] | | ** 54[illegible][18] | **535[17] |
| | Creatinine (g.) | Schiz | *287[14] | | ** 419[14] | **442[14] |

* [illegible]

** [illegible] lues from the

N.B. Raised figures represent number of cases

values between the normal and the schizophrenic men. This negative finding has also been noted in younger groups of subjects (Hoagland, *et al.*, 1953). Ageing apparently does not affect the lymphopenic response to ACTH, as both Solomon and Shock (1949) and Pincus (1950) have found.

In regard to the 17-ketosteroid/creatinine ratios (Table III) the basal levels of the patients are higher than those of the

normal subjects as is true in the younger groups studied by Pincus *et al* (1949) and Hoagland *et al* (1953) The response to ACTH is an increase in the normal subjects and a decrease in the elderly schizophrenic patients There are, however, no statistically significant differences between the normal subjects and the patients either in the control values or in the trends after the injection of ACTH A similar lack of differences in reactivity has been noted between young normal and schizophrenic men (Pincus *et al*, 1949)

The difference in trends between the normal and schizophrenic men may be due as has been attributed in younger subjects (Pincus *et al* 1949) to a factor of adrenocortical dysfunction It may also be related in this older group to the difference in the initial levels between the two groups of subjects While in the younger subjects there is no relationship between the basal values and the absolute change at four hours in this group there is a negative correlation of high value ($r = -0.70$) The normal subjects with their initial low values show increases at four hours, while the patients with their initial high values show decreases This relationship holds good as well for the 17 ketosteroid values alone as for the creatinine ratios

The basal values for the neutral reducing lipids or cortins (Table III) show a significantly higher level in the elderly schizophrenic patients While the normal subjects show a greater rise after ACTH than do the patients the trends are not significantly different This is true for younger subjects also (Pincus *et al* 1949) Again the slightly greater reactivity of the normal group may be due at least in part, to the difference in the initial levels There is a negative relationship ($r = -0.47$) between the initial values and the absolute change at four hours, so the normal subjects, initially lower, exhibit greater increases than the patients

In the values for the uric acid/creatinine ratios (Table III) there is no difference between the initial levels of elderly normal and psychotic men After the administration of ACTH there is an increase in the excretion in both groups

which is greater in the normal group than in the patients, but not to a statistically significant degree In younger individuals the normal subjects show a significantly greater response (Hoagland *et al*, 1953)

The greater reactivity noted in the response of the normal subjects is not due to any influence of the initial level No relationship can be demonstrated between the control levels and the magnitude of the change at the four hour reading

The sodium/creatinine excretion data in Table III show identical basal values for the two groups of subjects In younger subjects, patients usually excrete more sodium (Pincus *et al*, 1949) Their response to the injection of ACTH is distinctly different The normal subjects show a steady rise, more marked in the first two hours, the final level reached being an increase of 65 per cent In the case of the patients, there is an insignificant rise at the two hour reading and then a decrease in excretion to a point slightly below the initial level At both the two and four hour points the response of the normal subjects is statistically significantly greater than that of the patients A similar lesser responsivity is characteristic of young schizophrenic patients also

Again the responsivity of sodium to ACTH is independent of the initial level and since both groups have the same mean basal values the difference in trends is therefore more striking

Turning next to the data on potassium creatinine ratios (Table III) we see that in the basal levels the patients excrete a lesser amount than the normal subjects The reverse is true in younger subjects (Hoagland *et al*, 1953) After the administration of ACTH the excretion is increased in both groups, but significantly more in the normal subjects In this respect again the greater response of the elderly normal subjects as compared with the patients is a reflection of what has been noted in younger subjects (Pincus *et al* 1949) The initial level plays no role in the greater responsivity of the normal subjects there being no relationship between the control values and the amount of change at the four hour point

The phosphate/creatinine values (Table III) show that the normal subjects excrete twice as much inorganic phosphates on a creatinine basis as do patients, a statistically significant difference After ACTH the normal subjects show no increase but rather a mild decrease, the patients show a striking increase This difference in trends is statistically significant

The lesser excretion of phosphates, in the basal state, in schizophrenic patients is a consistent phenomenon throughout the various age decades (Hoagland *et al* 1953)

The excessive phosphate excretion of the patients after the injection of ACTH has also been noted in the younger groups (Hoagland *et al* 1953) To what extent this is a feature inherent in the schizophrenic psychosis is however, a question Like the 17 ketosteroids and the cortins there is a negative relationship between the initial levels and the amount of change after ACTH the correlation coefficient being −0 64 For phosphate values alone $r = -0\ 55$ Since the normal subjects have the high initial levels they show little effect from ACTH while the schizophrenic patients with low control values show increases The greater reactivity of the patients to ACTH may thus be just a reflection of their initial levels

In normal men there has been noted in all the urinary metabolites a decrease in excretion values with advancing age (Pincus 1950 Kirk 1949 Hamilton and Hamilton 1948, Kowalewski 1949) A point of interest is whether ageing affects schizophrenic patients in this regard as much as normal individuals In Table IV we have shown the mean values for the various constituents in young and old normal and schizophrenic groups

In order to minimize the intra individual variation the control values for several tests have been grouped for each subject and an individual mean taken

The results of the change from the young to the old group are expressed on a percentage basis in the last line

Several points are of interest The creatinine and phosphate values are lower in the patients consistently The sodium and

Tab

MEANS OF BASAL VALUES FOR URINARY CONSTITUENTS F

| Age group Years | Creat (g 24 hr) | | 17 KS (mg/hr) | | Cortin (mg/24 hr) | |
|---|---|---|---|---|---|---|
| | N | S | N | S | N | S |
| 19–30 | 1 80$^{56}$ | 1 C3$^{109}$ | 0 54$^{133}$ | 0 70 | 3 0? | 2 29 |
| 60 90 | 1 06$^{43}$ | 0 87$^{69}$ | 0 10$^{42}$ | 0 17 | 2 11$^{31}$ | 2 43 |
| Per cent change from young to old | −41 | −47 | −65 | −70 | −30 | + |

N B Raised figures represent number of tests

potassium values are higher in the young patients than in the young normals but not in the old patients The cortin values are lower in the young patients than in the opposing group but there is no essential difference between the two groups in the old population The uric acid values are essentially the same for the two groups for corresponding ages The percentage changes show that with increasing age, decreasing excretion appears in approximately the same proportion in the normal and schizophrenic groups in the creatinine, 17 ketosteroids uric acid and phosphates The cortins show no decrease in the patients an indication possibly that this portion of the adrenal cortex is functioning at a low level consistently throughout life The sodium and potassium decrease more in the patients than in the normal subjects primarily because of their higher values in youth Thus it is only in the corticosteroids and minerals that advancing age affects the schizophrenic subject differently from the normal subject

It is evident from the percentage changes that ageing could not be expressed with the creatinine ratios since with the exception of the 17 ketosteroids the creatinine decrease is sufficient in magnitude to nullify or even reverse the tendency to a decreasing excretion with age

...mal (N) and Schizophrenic (S) Men at Two Age Levels

| Uric Acid (mg/min) | | Sodium (mg/hr) | | Potassium (mg/hr) | | Phosphate (mg/min) | |
|---|---|---|---|---|---|---|---|
| N | S | N | S | N | S | N | S |
| [illegible] | 0 30[illegible] | 100[illegible] | 306[illegible] | 167[illegible] | 197[illegible] | 0 34[illegible] | 0 20[illegible] |
| [illegible] | 0 29[illegible] | 138[illegible] | 134[illegible] | 112[illegible] | 68[illegible] | 0 36[illegible] | 0 17[illegible] |
| −[illegible] | −44 | −27 | −66 | −33 | −65 | −33 | −35 |

A further point of interest is the question as to whether schizophrenic patients show a change in their reactivity to ACTH as they grow older Previous studies by Pincus (1950) and Solomon and Shock (1949) on ageing in normal men show that the adrenocortical responsivity is essentially unaffected, although the latter authors observed some deficiency in the renal handling of the uric acid Analysis of the data for young and old normal and schizophrenic men bears out this conclusion for patients also In Table V are shown the percentage changes from the basal values four hours after the injection of ACTH in young and old men of the normal and psychotic groups It is evident that for the normal subjects the only marked changes in ageing are a lesser reactivity in the excretion of uric acid and in potassium In the patients, as a result of senescence there is a moderate increase in the potassium excretion and a decrease in the phosphate excretion

We may conclude therefore, that on the whole, schizophrenic patients show the same degree of reactivity to ACTH in old age that they do in youth and that whatever aspects of lesser responsivity they exhibit are preserved throughout life

Interrelationships between the effects on the various functions studied have been notably lacking It seems that

each function operates independently of the others. How much of this is dependent on technical variation and how much is due to inherent differential effects of adrenocortical reactivity upon the various target organs or physiological systems is impossible to assess. It does, however, make it difficult to determine causal relationships.

**Table V**

MEAN VALUES FOR PER CENT CHANGES FROM BASAL LEVELS IN URINARY METABOLITES/CREATININE RATIOS FOUR HOURS AFTER THE INJECTION OF 25 MG ACTH IN ELDERLY NORMAL (N) AND SCHIZOPHRENIC (S) MEN

| Age Groups | 17-KS / Creat | | Cortin / Creat | | Uric Acid / Creat | | Sodium / Creat | | Potassium / Creat | | Phosphate / Creat | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Years | N | S | N | S | N | S | N | S | N | S | N | S |
| 19-30 | +25 | 0 | +21 | 13 | +64 | +11 | +50 | 0 | +124 | + 7 | −10 | +73 |
| 60-69 | +25 | 8 | + 9 | +1 | +20 | +18 | +65 | 10 | +95 | + 3 | − 7 | +54 |

The data on the response to glucose is shown in Table VI. So far as the blood sugar is concerned, the trends in the two groups are quite similar except that the patients show a significantly greater elevation at the one hour reading than do the normal subjects. This decrease in tolerance is characteristic of schizophrenic patients also (Pincus *et al.*, 1949; Freeman and Elmadjian, 1950).

One of the usual concomitants of ageing is a tendency to show a diabetic-like blood glucose trend, whether sugar is given by mouth (Smith, 1948) or intravenously (Smith and Shock, 1949). In young normal subjects the thirty minute value is usually higher than the sixty minute reading. In old normal subjects the thirty minute reading is higher than that of the younger individuals and the sixty minute value is further elevated over the thirty minute one. In young schizophrenic patients the sixty minute reading is usually higher than the thirty minute level, and in old schizophrenic subjects the trend is exaggerated. The factors causing the reduction in glucose tolerance of the schizophrenic patients are unknown.

One possible cause has been thought to be emotional tension which has acted via the hypothalamus on the anterior pituitary, stimulating the production of corticotrophins and adrenocortical steroids resulting in gluconeogenesis from

Table VI

Values for Blood and Urinary Constituents in Elderly Normal and Schizophrenic Men During Exton Rose Glucose Tolerance Test

| | Variable | Subject | Basal | ½ hr | 2 hr | 3 hr |
|---|---|---|---|---|---|---|
| Blood | Sugar | Norm | 104 | 149 | **163 | 111 |
| | | Schiz | 91 | 151 | **176 | 95 |
| | Eosinophils | Norm | 183 | 170 | 165 | 14[illegible] |
| | | Schiz | 128 | 108 | 102 | 106 |
| | Lymphocytes | Norm | 2032 | 2071 | 2185 | 2539 |
| | | Schiz | 2913 | 2083 | 1290 | 2813 |
| Urinary Excretion per 24 hours | 17-KS (mg)/Creatinine (g) | Norm | 4.23 | | 4.06 | 3.84 |
| | | Schiz | 5.15 | | 4.51 | 3.67 |
| | Cortin mg/Creatinine g | Norm | *3.30 | | 3.60 | |
| | | Schiz | *3.13 | | 2.56 | |
| | Uric Acid mg/Creatinine g | Norm | 345 | | 441 | **501 |
| | | Schiz | 497 | | 507 | **430 |
| | Sodium mg/Creatinine g | Norm | 3067 | | **3[illegible]13 | **2354 |
| | | Schiz | 3[illegible]82 | | **2700 | **[illegible]782 |
| | Potassium (mg)/Creatinine g | Norm | 2500 | | 2465 | **2529 |
| | | Schiz | 1844 | | 159[illegible] | **1131 |
| | Phosphates mg/Creatinine g | Norm | *476 | | **497 | **403 |
| | | Schiz | *481 | | **503 | **370 |

* = statistically significant differences between basal values in the two groups

** = statistically significant differences between the changes in the 2 or 3 hour values from the basal levels between the two groups

N.B. Raised figures represent number of cases

glycogen. In older people it may be that the elimination of sugar through the kidney and not the production is at fault (Shock 1946). At any rate both the factors involved in the psychosis and in ageing result in the maintenance of a reduced sugar tolerance in the elderly psychotic group.

In the eosinophile response (Table VI) the normal subjects again have a higher (but not significantly so) initial value

The trend is steadily downward over the four hour period There are no significant differences between the two groups of subjects in this regard A similar lack of differentiation has been noted in younger groups (Hoagland *et al* 1953)

Ageing plays no role in the eosinophil response the decrease being of the same magnitude roughly at all ages (Solomon and Shock 1949) In view of the fact that the diurnal variation of eosinophils results in a decrease of approximately 21 per cent in the morning hours (Stevenson Metcalfe and Hobbs 1953) and that the decrease seen above is about this magnitude the effect of the glucose as a cause of the eosinopenia is somewhat doubtful

The values for the lymphocytes are shown in Table VI After the ingestion of glucose the lymphocytes show a drop in values at the half hour reading which continues for the next half hour and then returns almost to the original values by three hours These trends are similar in both groups of subjects indicating no difference in response This is true also for younger groups (Pincus *et al* 1949)

The lymphocyte response is in the main a mirror image of the blood glucose tolerance trend In the time relationship to the glycemic curve the lymphocytes react more quickly than the eosinophils

In the case of the 17 ketosteroid/creatinine ratios (Table VI) the patients initial values are higher (but not significantly so) than those of the normal subjects a finding also noted in younger subjects (Pincus *et al* 1949) In both normal and schizophrenic subjects the values show a downward trend more steeply for the patients so that the levels at the three hour reading are the same In neither group is there any indication of adrenocortical stimulation The greater decrease in values in the patients may be due to their initially higher values since there is a negative relationship ($r = -0.60$) between the basal value and the absolute change at the three hour point

So far as ageing is concerned the response to glucose diminishes with increasing years The younger normal

subjects, but not the patients, show an increase at the one- and three hour points while, as we have seen, both of the older groups show a decrease (Pincus, *et al*, 1949)

The cortin/creatinine ratios, again as in the case of the ACTH data, show significantly higher values in the case of the patients In the younger groups, the reverse is usually true (Pincus *et al*, 1949) There is no response to the glucose, there being a decrease at the one hour reading in each case, the trend being the same for both normal and psychotic groups Ageing seems to play no role in the response to glucose in this function, the younger groups being equally unreactive to glucose (Pincus, 1950)

The uric acid values in Table VI show initially higher levels of uric acid excretion in the patients, a trend noted in younger subjects (Hoagland *et al*, 1953) After the ingestion of glucose the normal subjects show an increase in excretion at the one-hour and three hour readings The schizophrenic patients at the one hour show a slight upward trend and at three hours a downward trend At the three hour point, the difference between the increase in the normal subjects and the decrease in the schizophrenic patients is statistically significant This lesser responsivity of elderly schizophrenic patients has been noted also in younger schizophrenic patients (Pincus *et al*, 1949) The initial levels of uric acid/creatinine excretion bear no relationship to the response following glucose so that any difference between the trends in the normal and psychotic groups cannot be attributed to the differences in their initial levels

In the values for sodium/creatinine ratios (Table VI) the patients have initially higher levels than the normals but not to a statistically significant degree During the three hour test period the normal subjects show on the whole, a steady mild upward trend The patients show a marked fall at one hour, with a subsequent levelling off of the values for the next two hours The difference in trends between the normal and schizophrenic groups is statistically significant at the one hour and three hour readings This difference in trends between

The trend is steadily downward over the four hour period. There are no significant differences between the two groups of subjects in this regard. A similar lack of differentiation has been noted in younger groups (Hoagland *et al.*, 1953).

Ageing plays no rôle in the eosinophil response, the decrease being of the same magnitude roughly at all ages (Solomon and Shock, 1949). In view of the fact that the diurnal variation of eosinophils results in a decrease of approximately 21 per cent in the morning hours (Stevenson, Metcalfe and Hobbs, 1953) and that the decrease seen above is about this magnitude, the effect of the glucose as a cause of the eosinopenia is somewhat doubtful.

The values for the lymphocytes are shown in Table VI. After the ingestion of glucose the lymphocytes show a drop in values at the half-hour reading, which continues for the next half hour and then returns almost to the original values by three hours. These trends are similar in both groups of subjects, indicating no difference in response. This is true also for younger groups (Pincus *et al.*, 1949).

The lymphocyte response is, in the main, a mirror image of the blood glucose tolerance trend. In the time relationship to the glycemic curve, the lymphocytes react more quickly than the eosinophils.

In the case of the 17-ketosteroid/creatinine ratios (Table VI) the patients' initial values are higher (but not significantly so) than those of the normal subjects, a finding also noted in younger subjects (Pincus *et al.*, 1949). In both normal and schizophrenic subjects the values show a downward trend, more steeply for the patients, so that the levels at the three-hour reading are the same. In neither group is there any indication of adrenocortical stimulation. The greater decrease in values in the patients may be due to their initially higher values, since there is a negative relationship ($r = -0.60$) between the basal value and the absolute change at the three hour point.

So far as ageing is concerned, the response to glucose diminishes with increasing years. The younger normal

subjects but not the patients, show an increase at the one- and three-hour points while, as we have seen, both of the older groups show a decrease (Pincus, *et al*, 1949)

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the two groups is similar to that found in younger subjects (Pincus *et al*, 1949) but it may be, in part, due to the difference in the initial levels There is a negative relationship ($r = -0.46$) between the basal values and the absolute changes at three hours, so that the higher values of the patients tend to show the greatest decreases

The potassium/creatinine values (Table VI) again show the normal subjects to have somewhat higher values than the patients although the difference is not statistically significant During the three hour period the normal subjects show a slight decline in excretion values while in the patients the decrease is quite marked to a value approximately 40 per cent below the initial level This difference in trends is significantly different between the two groups

The initial levels have some influence in the degree of response at three hours There is a negative relationship ($r = -0.40$) between the control levels and the absolute change at the three hour reading On this basis the normal subjects with initial higher values would be expected to show the greater decrease That they do not emphasizes the real nature of the difference in trends between the two groups

The values for the phosphate/creatinine ratios (Table VI) show the characteristically low initial levels of the schizophrenic patients After the ingestion of glucose in the normal subjects there is a mild rise at the one hour reading with a subsequent fall below the initial level at the three hour reading In the patients there is a marked increase at the one hour reading to the level of the normal subjects and then a marked fall almost back to the original level The trends at both one and three hours as compared with the initial levels are statistically significantly different between the two groups of subjects

It would seem then, that in the normal subjects the ingestion of glucose produces a conservation of phosphate in the body so that less is excreted In the patients on the other hand more phosphate is excreted Here the difference in initial levels between the two groups of subjects may play a

role in this varying type of reaction since there is a highly negative relationship (r= −0 91) between the initial level and the absolute change at the three hour point Thus, the patients with their low values would show a rise and the normal subjects with their higher values would show a fall Again as in the case following ACTH injection, we may say that the initially low level of urinary phosphates is a characteristic of schizophrenia but the reaction to the stress on stimulus is largely dependent on that level and may have little bearing on the dysfunction in the psychosis

Analysis of the data for inter relationships between various functions again, as in the case of ACTH proved disappointing No significant co variance was noted between any of the variables studied

## Discussion

It may be said in general that elderly schizophrenic patients exhibit the same differences from elderly normal subjects that young patients do from young normal individuals There is less tendency in the old to show the increases in ketosteroid and corticoid excretion than in the young but the differences in responses in the mineral elements are essentially identical throughout the life span It is probably true that the renal mechanisms in the aged play a greater role in the responsivity of the organism to the two stress situations employed, but this fact does not obviate the persistence of differential responses between the normal and schizophrenic subjects

The maintenance of these abnormalities in the ageing schizophrenic group poses an interesting problem as to whether the adrenal cortex really suffers from exhaustion over the many years in which these patients have experienced a profound psychotic reaction It may be however, that the sheltering of such patients from the stresses of existence out side may minimize the stimulating effect upon the adrenal cortex of the constant emotional reaction In any case, it is evident that the physiological defects in these elderly schizophrenic patients whatever their cause, have no deleterious effects upon the life expectancy of such subjects

## Summary

A study was made of the adrenocortical responsivity of 34 elderly normal men and 33 aged schizophrenic patients hospitalized for an average period of thirty years. Following the injection of 25 mg. of ACTH and the ingestion of glucose the blood and urinary functions under investigation showed the same degree of dysfunction as had been noted in young schizophrenic subjects.

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## DISCUSSION

*Nicolaysen*: If you observe the urinary composition from the moment you wake up and throughout the day, you observe cyclic changes in many constituents. The concentration may be nearly doubled from say seven o'clock up to eleven, and then it falls back again: that's in experiments with no food or water intake.

*Freeman*: Well, diurnal variation is something we have to take into consideration always. As a matter of fact there isn't too much good literature on this particular point, because individuals have been studied at various times, but most of the experimenters have not got the data for the exact hours in which our tests were performed. One can say that although diurnal variation played a rôle in modifying the response

o ACTH or glucose, at least the same factors were present in both normals and schizophrenics, and from that point of view it doesn't alter our facts

*Nicolaysen* A long time ago I studied these cyclic changes in the urinary composition Some years later parallel studies were reported in schizophrenics The results were the same as in normal subjects, never ... due to the schizophrenic state

ontrol

fect of

h less

response than is assumed

*Nicolaysen* I am returning to my point about controls, because in those days it was maintained that pituitrin influenced tubular re absorption of fixed bases However, placing my curves over the curves from the experiments in which pituitrin had been injected showed that the two sets of observations were identical. Obviously your conclusions would not necessarily be invalidated, but they might be somewhat modified

*Freeman* Yes, I think that has probably not been taken into account sufficiently in all the studies on ACTH Practically nobody in the ... done anything on this

patients have on the whole shown some insulin resistance This was also noted by Meduna that they drop down to a lesser degree in a given time if you take rapid intervals, and on the whole they don't show as quick or as great a response to a given dose of insulin as normal individuals do

*Olbrich* You see the same thing in senile dementias or in any type of neurological lesions with focalizing sites

*Freeman* It is undoubtedly true that reduced glucose tolerance is found in other types of psychosis, because we have studied manic

creatinine

*Freeman* This ratio is probably not so important in young individuals because the creatinine doesn't vary with the ACTH or glucose but it certainly does vary in old people

*Shock* A study is just being completed in our laboratory by Drs Silverstone and Brandfonbrener, who have been investigating the response of blood sugar to insulin in normal old people We have found that older individuals uniformly show a reduced response to insulin In these experiments a standard amount of insulin was administered intravenously along with 25 g of glucose also given intravenously

Blood sugar levels were det . . . of two hours In all n the blood of the olde . . . was for the young and middle aged groups Since we also . . . and intravenous glucose tolerance curves . . . to show that . . . schizophrenics received . . .

*Freeman* No

## GENERAL DISCUSSION

*Tunbridge* I think that Prof Brull and Prof Verzár suggested almost complementarily that any assessment of ageing . . . function, and that the . . . nd in . . . Dr . . . concurrently? We can . . . afterwards

*Comfort* I have been . . . laboratory stocks such as . . . and longevity in man is . . . rather difficult to specify . . .

. . . a point I want to make is . . . Pearson (*Biometrika* 1901 1 50) a long time . . . studied the sib sib and parent . . . I think only ab . . . at which one would . . . this type of pattern is what you would expect in any case where the . . . than . . .

. . . mig . . . gen . . .

eight generations of *Drosophila subobscura* They were bred in each generation from the eggs laid by flies after the thirtieth day of imaginal life The object of the experiment was to try to find the same thing as Dr Lansing found in rotifers and to see whether the life-span of the offspring of old parents decreased cumulatively It didn't do so in this experiment there is no significant trend in those figures over eight generations occupying about a year But even more interesting is the stability of the life span In each case we were breeding from the longest lived



*Drosophila* On the right you have the survival curves under the same conditions for the two reciprocal hybrids, $\frac{K}{B}$ and $\frac{B}{K}$ I think that is rather an instructive example of the effect of heterosis, of induced hybrid vigour, on the life span The degree of increase—I did superimpose the pictures—is almost exactly comparable quantitatively to the degree of decrease in life span which was obtained in the rat experiments

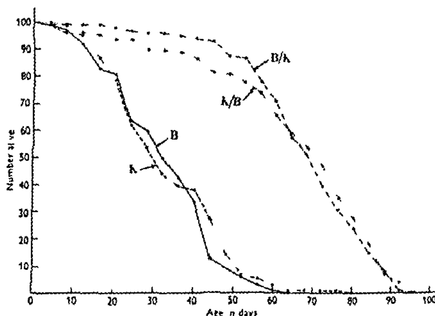



FIG 2 (Comfort) *Drosophila subobscura* Survival curves of inbred lines B and K and of the reciprocal hybrids between them (sexes combined )

Clarke, J M and Maynard Smith, J (1955) *J Genet*, 53 172

which Dr McCay published There was approximate doubling of life-span and no change in the slope of eventual mortality, what has been done is to push the whole thing over with a much longer plateau

I wanted to *show that as an indication of the extremely large effect* which can be produced by a genetic factor in this case heterosis Now had we started with that particular hybrid cross, and inbred from it, I have no doubt that over five or six more generations we should slowly push the life table back again to the position of curves B and K the equilibrium position which we have when we have a line that has been partially inbred for some time That is rather an awful warning in so far as these laboratory stocks are concerned We can t, I know, go

here into the nature of heterosis but I think it is fair to point out that while Dr Maynard Smith has shown (*J Genet* 1950 52 152) that in this particular case an orthodox Mendelian explanation a chromosomal explanation is necessary vigour is a very heterogeneous thing and there are quite a number of instances in which an apparent hybrid vigour can be induced by non chromosomal means even I believe by cross suckling or by transplantation of fœtuses from one animal to another

There is only one remaining point that I should like to make and that is that we are working with stocks that have been in the laboratory for some time and the wild *Drosophila* which are not wild type but are actually caught in the wild have often been noticed to have a great deal longer life-span than our domestic *Drosophila* The longest lived one which Dr Maynard Smith has observed had a life span after catching—it may have lived already for some days before that—which suggested that it would live for about as long as those vigorous hybrids Of course we have no evidence as yet that heterosis hybridization can produce a greater life span than is found in the wild animal. I just want to stress the dangers into which we run if we do ageing experiments on inbred lines which are showing the degree of stability and the degree of inbreeding depression which we found in our *Drosophila*

*Brull* I am glad that you have stressed the genetical influence which is so important in ageing If you can say that in small animals genetical influence is so great you could go further in mankind and say that genetics in mankind not only influence duration of life but also the form of ageing In this field much can be done and should be done If I may make a suggestion for future research and for future meetings, it ould be to have meetings of clinicians who have studied the special eld of heredity in man and cross clinicians with geneticists

*Comfort* And thereby obtain some hybrid vigour?

*Lansing* I think Dr Comfort has raised a very interesting point in onnection with hybrid vigour and I m very much impressed with the tudies on *Drosophila* that attempted to repeat the work that we did with rotifers and did not succeed I wonder if there is a fundamental difference in our material? We of course have flirted with the possibility that there are differences The rotifer ceases all cell division in the embryo the newly hatched rotifer has all the cells it is going to have they merely get larger If I m not mistaken in *Drosophila* division in t [illegible] nues and so we may have there a very fundamental [illegible] of material The rotifer eggs have time [illegible] while in *Drosophila*

[illegible] e part of the [illegible]

know to what [illegible]

ent [illegible] ght be able to tell us [illegible]

[illegible]

of ageing [illegible] definition

going, said "Ah ain't goin' nowhere, Ah'm comin' away from some place".

*Shock:* Does a definition of ageing have to be limited to decreasing functions?

*Franklin:* Change of function, surely.

u use the word

You have to

*Coudry: I have a definition. Ageing is change with time in the life cycle.*

*Krohn:* When would you define somebody as having reached adulthood?

*Lansing:* When he has reached maximum size.

*Gross.* There should be a distinction between development and ageing.

existence to describe a process human beings don't like, a deteriorative process, and I take it that it is senescence with which we are concerned

don't feed at all and appear to deplete their reserves, and we have some animals, as has already been mentioned, which die automatically after breeding, possibly from depletion, we have the elephant, which, I'm told, dies in the wild state when its teeth wear out. I have no doubt that if you gave the elephant false teeth, he would die of some other senescent process eventually. I don't want to speak out of turn, but

very degenerate character who starves to death in the twenty four hour period that he is busy fertilizing

*Comfort* But you can t prove, although I think it a highly improbable, that even the mammal does not die from depletion of something We know so little about the processes which actually operate I really don t see how we can discriminate

*Lansing* Well I rather hope that we can It seems to me that there are (in spite of my declaration of faith yesterday) in biology a number

wind up dead sooner or later That s what I mean by senescence The maturation of the embryo, the new born child the adolescent, the changes with time prior to maturation to me are not senescence

*Cowdry* Yours is the downswing of life then

*Lansing* Yes *after* adulthood has been reached I can t define

phylum to phylum

*Lansing* I m not prepared to agree to that I think we have special [illegible] death but not all death is due to senescence [illegible] you would know about [illegible] enzymes are [illegible] life accord ing to our orthodox [illegible] knowledge, we could give a chemical definition of [illegible] then work backwards and see how much we know about the vital organs *in old age* This might be a useful approach

*Lansing* The declaration of faith I made yesterday stems in part [illegible] types of survival curves that Dr Comfort showed us [illegible] was virtually identical with the sur [illegible] scale is a little [illegible] projects the [illegible] xcept that we [illegible] for mice and

was [illegible]

*Comfort* Raymond Pearl plotted a surv [illegible] which was again the same shape! [illegible]

and, asked by someone, when he was going at full speed, where he was going, said "Ah ain't goin' nowhere, Ah'm comin' away from some place".

*Shock:* Does a definition of ageing have to be limited to decreasing functions?

Change of function, surely.

You use the word ... You have to

time in the life cycle.

hood?

*Lansing:* When he has reached maximum size.

*Gross:* There should be a distinction between development and ageing.

process, and I take it that it is senescence with which we are concerned

exhaustion of enzyme systems in cells which don't divide might provide us with something of the kind. But if we do accept, as I suggest we should, the idea of senescence simply as the increasing liability to die

senescent process eventually. I don't want to speak out of turn,

ageing process,

scence simply as

animals have a

determinate life-span instead of an indeterminate one.

tion of life tables might be an index as to what you were doing to a process, but if you are going to explain ageing as a process I think ultimately you have to look at individuals and perhaps the best way is to look at them from different points of view and at different levels of organization I doubt if it would be possible to formulate a definition of ageing that would be acceptable to everybody and would cover all the aspects of the problem as it now stands

*Franklin* Xavier Bichat, I think, defined life as the sum of the forces which resist death Some of us find that ageing is death taking small bites

* * * *

*Tunbridge* I think we shall have to accept ageing as a chronological process

To summarize this very interesting colloquium Dr McCay has emphasized that the conditions of experiments however well planned may be artificial for whatever animal or insect or fish we select, and that even in animal experiments disease processes must be taken into account

Heredity was mentioned by Prof Medawar and Dr Comfort It obviously comes into the story, but the work seems to show that there may be fallacies in some of the current views We do not know to what extent there is a true species difference Differences may be due to environmental conditions as Prof Krohn mentioned for the ovary—and not necessarily to chromosome determination Throughout I think we have come across the difficulty of assessing environmental factors They are difficult to control almost impossible in man and we tend to deal with end results instead of looking for primary causes, in man because of the long life cycle we may have been too obsessed with the former We agreed that although pathological arterial changes occur, they are not specific for any age group it is a question of severity, which again may be due to the passage of time

There was some difference of opinion among the pathologists as to [illegible] tell the age of a cell Some agreed that with the [illegible] possible I think that is [illegible] We

[illegible]

[illegible] for [illegible] At the

[illegible]

*Shock* I think the argument that because two different phenomena can be made to fit the same mathematical formulation they have common processes behind them is an extremely hazardous one

*Lansing* I said only that it's a possibility I'm not prepared to say that we have as many kinds of protoplasm as we have species I think there is a common protoplasm with basic properties of multiplication and growth, decline, irritability and so on, varying in detail not in principle

*Comfort* Does that imply that you think that senescence is probably inherent in all metazoa?

*Lansing* In so far as we can observe it, it is a natural phenomenon I would like to avoid the implication that because it may be inherent it must be inevitable

*Comfort* I was wondering about the case of the sea anemone At Edinburgh there were sea anemones which were in captivity for about ninety years without any change in shape they apparently grew new

[illegible]

thing for *Hydra*

*Lansing* But for instance the protozoa were held to be contradictory to this hypothesis until the work was done and then we found that there is senescence in protozoa just as there is in any other species, unless a particular biological process intervenes, autogamy or conjugation In the absence of the latter, *Paramecia* go on to die, following survival curves very much like those for man

*Shock* I would agree that protoplasm is probably fundamentally much the same stuff although we know that various tissues develop different functions, so that their enzyme systems must vary quite widely between different cells in the same animal To that extent, I would agree that per' [illegible] lose its ability to [illegible] metabolic processes [illegible] ageing process But it seems to me [illegible] study, but unfortunately [illegible] in two new cells so that [illegible] th the problem of studying more complex animals or tissue using both biochemical and physiological techniques Since changes in the environment of the cell, pro- [illegible] will often result in alterations [illegible]

might be, though not doing at seventy what we did at twenty I think also we were given hope (in the not too distant future) of a highly selective human Ford service—so that when the doctor diagnoses our organs as being tired or out of date we should be able to apply for a [illegible] decoked artery, or a refined lung

[illegible] express on your behalf our
[illegible] maximum
We have [illegible] m the
[illegible] have been [illegible] t time
[illegible] of all [illegible] he first
[illegible] nological aspects
With it has [illegible] ntion We have
been honoured by the presence as host [illegible] e Foundation, of
[illegible]

scheme by the Ciba Foundation designed to encourage and develop research on the problems of ageing

increased the expectancy of life, but such an influence was not apparent

Prof. Lewis stressed the need for longitudinal studies. The statistical method has its value, but it also has its limitations. We need to have longitudinal studies, because only so can we correct *many of our early*

for the maintenance of such peaks. We did not quite agree that there was necessarily a uniform steady percentage decrease from the peak performance with age; this would of course introduce a further variant.

When we come to studies *in man* we are up against the question of environment, which in turn introduces the factors of disease and of impaired reserve. Prof. Christie showed very clearly that in the lungs there is a decrease in functional capacity, whether due to the onset of

function would appear to show *some decrease with age* which is not entirely conditioned by the environment, in this case the fixity of the chest wall.

possibilities in his experiments on freezing.

*I end on an imaginative but provocative note.* We know that when an

biophysical dissolution?

# SUBJECT INDEX

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